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La Revue *Proceedings of the Nova Scotian Institute of Science* publie les travaux de l'Institut, des articles originaux et des notes avant trait aux sciences naturelles aux Provinces atlantiques, surtout dans les domaines de la biologie et de la géologie. Les manuscrits peuvent être en anglais ou en français, et seront soumis à l'avis d'experts avant d'être acceptés.

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EFFECTS OF *Pisolithus arhizus* AND OTHER ECTOMYCORRHIZAL FUNGI ON *in vitro* ROOTING OF *Pinus monticola* ADVENTITIOUS SHOOTS[1]*

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Micropropagation of conifers permits the production of large numbers of selected genotypes but in *Pinus monticola* as well as several other systems, this process is limited by the difficulty of obtaining roots. The potential of ectomycorrhizal fungi to induce and improve adventitious root formation on in vitro micropropagated shoots was investigated.

Preliminary trials on de-rooted seedlings showed 100% rooting with *Laccaria bicolor, Rhizopogon rubescens* and *Pisolithus arhizus* while rooting on controls was at 79 %; however only *P. arhizus* had a significant effect on the rooting of micropropagated shoots.

Co-cultivation with *P. arhizus* in a medium amended with 0.3 µM 1-naphthaleneacetic acid (NAA) gave the best rooting percentages (57%).

Thin layer chromatography, high-performance liquid chromatography, and gas chromatography/mass spectrometric analysis of the broth from a *P. arhizus* culture in NAA amended media indicated the presence of hydroxylated NAA metabolites.

La multiplication végétative *in vitro* rend possible la production d'un vaste nombre de génotypes choisis. Cependant, pour le *Pinus monticola* et d'autres essences diverses, cette méthode de production se trouve limitée par la difficulté d'obtenir des racines. Les auteurs ont examiné le potentiel des champignons ectomycorhiziens à activer et à améliorer la formation de racines adventives sur des tiges micropropagagées.

Une étude exploratoire à partir de semis déracinés montre qu'il est possible d'obtenir un enracinement de 100% avec le *Laccaria bicolor*, le *Rhizopogon rubescens* et le *Pisolithus arhizus*, comparativement à un enracinement de 79% avec les échantillons-témoins. Toutefois, seul le *P. arhizus* exerce une forte influence sur l'enracinement de tiges micropropagagées. La coculture avec le *P. arhizus* dans un milieu modifié par de l'acide naphtalène acétique (ANA) à raison de 0,3 µM a donné les meilleurs taux d'enracinement (57 %).

Les analyses par chromatographie sur couche mince, par chromatographie liquide haute performance et par couplage chromatographie en phase gazeuse-spectrométrie de masse, du bouillon de la culture de *P. arhizus* dans un milieu modifié avec de l'ANA révèlent la présence de métabolites d'ANA hydroxylées.

Introduction

Micropropagation of conifers permits tree breeders to produce large numbers of selected genotypes but in many conifer systems this process is limited by the difficulties in obtaining roots. A method for production of adventitious shoots from *Pinus monticola* Dougl. embryos and buds has been developed but various factors investigated, including auxin treatments, did not improve rooting over that which occurs spontaneously at about 20% (Lapp and Malinek 1988).

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* This paper is dedicated to the memory of K.A. Harrison.

Many ectomycorrhizal fungi release growth regulators such as: auxins, cytokinins, ethylene, gibberellins, vitamins and amino acids into the culture medium (Kraigher *et al.* 1991; Frankenberger and Poth 1987; Strzelczyk *et al.* 1986). The effects of ectomycorrhizal fungi on rooting of cuttings or derooted hypocotyls has been examined by several workers. Navratil and Rochon (1981) found that the addition of a *Pisolithus tinctorius* inoculum to the vermiculite-based medium enhanced root and shoot development of four poplar hybrids. *Laccaria laccata* added to the rooting medium stimulated root initiation in apple rootstock cuttings as compared with indole-3-butyric acid treated cuttings (Branzanti *et al.* 1984). Kim and Lee (1990) reported that inoculation with *P. tinctorius* enhanced rooting of *Quercus acutissima* Carr. cuttings taken from 2, 4 and 20-year-old trees. Gay (1990) observed increased rooting percentages with *Pinus halepensis* hypocotyls in the presence of *Hebeloma hiemale* and tryptophan or with culture filtrates from this same combination and suggested that these fungi may be useful to enhance *in vitro* rooting.

This study investigates the potential of the ectomycorrhizal fungi *Laccaria bicolor, Pisolithus arhizus, Rhizopogon rubescens* and *Wilcoxina mikolae* to increase adventitious root formation of *in vitro* micropropagated *Pinus monticola* shoots and examines the interaction between *P. arhizus* (Scop: Pers.) Rauschert (syn. *Pisolithus tinctorius* (Pers.)(Coker & Couch) and the synthetic auxin 1-naphthaleneacetic acid (NAA).

Materials and methods

Derooted hypocotyls After stratification in running water at 4°C for 1 week, seeds were surface sterilized for 25 min in 30% H_2O_2 with the addition of a drop of Tween 80 and placed on 1% w/v water agar for germination in darkness. Germinated seeds were transferred to 3% w/v sucrose solution solidified with 5% Gel-Gro (ICN Biochemicals, Cleveland, OH, 44128) in glass jars for 21 days in controlled environmental conditions of 16 h light : 8 h dark at 23°C. Light was supplied from both cool white flourescent and incandescent sources at a photon density of 120 $\mu Em^{-2}s^{-1}$.

Roots were excised from the seedlings 1.0 cm above the transition zone between root and hypocotyl. The base of each hypocotyl was wounded with a scalpel before being placed in a vermiculite:peat (9:1) substrate with 0.5% pine charcoal (Sigma Chemical Co., St. Louis, MO, Number C-4386) added. Three 7- mm diameter inoculum plugs were cut from the growing edge of a 14-day fungal culture and placed in the substrate near the base of the inserted seedling. Experiments were carried out in 25 x 150 mm glass tubes under the same controlled conditions as previously described.

Fungal Cultures Cultures were maintained at 20° C in darkness on modified Melin Norkrans medium (MMN) (Marx, 1969). The following cultures were tested in rooting bioassays: *Laccaria bicolor* (Maire) Orton FTK 862A*, *Pisolithus arhizus* (Scop: Pers.) Rausch FTK 820A, *Rhizopogon rubescens* (Tul.) Tulasne FTK 861A and *Wilcoxina mikolae* Yang & Korf (E-strain) FTK 849A.

Still liquid cultures of *P. arhizus* were prepared by placing ten 7- mm diameter inoculum plugs cut from the perimeter of an actively growing culture, in 50 mL of MMN in a 125 mL flask with or without the addition of NAA (50 mg L^{-1}), before autoclaving. Cultures were incubated for 21 days in darkness at 20°C.

Micropropagated Shoots Micropropagated *P. monticola* shoots, derived from mature embryos (Lapp and Malinek 1988) were harvested 13 weeks after hormone treatment and elongated for an additional 4 weeks on half strength Litvay's medium (Litvay *et al.*, 1981) modified to increase the iron concentration two fold. After each shoot was freshly cut on the diagonal and wounded at the base with a scalpel, it was inserted into the rooting medium. Rooting was carried out on half strength Gresshoff Doy medium (GD) (Sigma Chemical Co., St. Louis, MO). The pH was adjusted to 5.5 before autoclaving or the addition of the gelling agent (5% w/v Gel Gro). Tryptophan or indole-3-acetic acid (IAA) were filter sterilized and added to the medium after autoclaving whereas NAA was autoclaved in the medium. A fungal inoculum plug was introduced into the tube at the base of the slant. Agar (MMN) plugs were introduced into tubes that served as controls. Black paper collars were placed around the base of each tube to protect the medium from excessive light exposure. After 10 weeks, shoots were examined for root formation and rooted shoots were transferred to a vermiculite: peat substrate in a greenhouse mistbed.

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* Accession number to culture collection held at Forintek Canada Corp.

Experimental Design Statistical analyses were done using NWA Statpak (Northwest Analytical Inc., Portland, Oregon, Version 4.1). The Chi-square test for independence was used to determine significant differences among treatments in rooting studies. Differences in root numbers or root lengths were examined by analysis of variance followed by Duncan's multiple range test. A completely random experimental design was used for all experiments. All experiments were duplicated.

Chemical Analyses All solvents were high-performance liquid chromatography (HPLC) grade. Analytical thin layer chromatography (TLC) was carried out on Merck, Kieselgel 60 F_{254} (Merck, Darmsdat), aluminum sheets, 5 x 2 x 0.02 cm; compounds were visualized by exposure to ultraviolet light (254 nm) and by dipping the plates in a 5% aqueous (w/v) sulphuric acid, followed by heating at 200°C. HPLC analysis was performed with the following Spectra Physics instrument system: liquid chromatograph model SP 8100, equipped with a dual pump and autosampler, ultraviolet/visible detector (variable wavelength) model SP 4200, and computing integrator model SP 4200. A reverse phase RP-8 Spheri-5 column (5 μm particle size silica, 22 x 0.46 cm internal diameter, Brownlee Columns, Applied Biosystems Inc., San Jose, CA, USA) equipped with a NewGuard TM RP-8 guard column, was used with a gradient elution of methyl alcohol-water-acetic acid (100:99:1, 5 min: 40:9:1, 20 min), a flow rate of 2.0 mL min^{-1}, and detection at 270 nm. Under these conditions, the retention time for NAA was 4.0 min. Gas chromatography-electron impact mass spectrometry (GC-EIMS) analysis was performed using a Finnigan Model 4500 instrument, having the gas chromatograph equipped with an Ultra-2 fused silica capillary column (50 m x 0.32 mm; Hewlett Packard, Palo Alto, CA, USA), in splitless mode injection with splitter closed for 0.8 min, at 50-250°C; temperature gradient 4°C min^{-1}, and helium as the carrier gas (flow rate 40 cm sec^{-1}, measured at 150°C). Diazomethane (ethereal solution) was prepared from p-toluenesulfonylmethylnitrosoamide (de Boer and Backer, 1963). Methylation of the broth extracts, dissolved in ethyl ether (2mL), was carried out at room temperature.

Twenty one day old liquid cultures of *P. arhizus* were filtered through several layers of cheesecloth. The broth from NAA-amended and from non amended cultures was lyophilized and maintained at -20° C for further studies. The freeze dried broth was diluted with water to one tenth of the initial volume and extracted with ethyl ether. The ethereal extracts were dried (Na_2SO_4) and concentrated under vacuum at 35°C. The concentrated extracts were analyzed by TLC using the solvent system dichloromethane methanol (96:4, double elution), HPLC and GC-EIMS. Medium (MMN) containing NAA was also extracted and analyzed as described above.

Results and discussion

All of the de-rooted seedlings co-cultured with ectomycorrhizal fungi showed 100% rooting while 79% of controls rooted (Table I). Seedlings cultured in the presence of *P. arhizus* had a significantly greater mean number of roots formed per seedling than the other treatments. Total mean root length per seedling was greatest (9.2) where seedlings were cultured with *L. bicolor*, but did not differ significantly from that of *P. arhizus* co-cultured seedlings (7.0).

Contingency Chi-square analysis of rooting percentages of micropropagated *P. monticola* shoots showed that amending the media with NAA or the auxin precursor, tryptophan, did not affect rooting ($p<0.5$) (Table II). Fungal treatments did significantly increase rooting ($p<0.005$) with a total of 22% for all *P. arhizus* treated shoots while controls and shoots cultured with *L. bicolor* or *W. mikolae* had 9% rooting. The media x fungus interaction was significant ($p<0.05$) indicating that within each fungal treatment, differences could be attributed to media amendments. Little callus formation was observed on those shoots co-cultivated with fungi. Viable hyphae, which initiated new cultures when placed on MMN agar plates, were observed in the vicinity and often covering growing root tips (Fig 1). However, no true mantle or Hartig net structures were visible in squash mounts of short roots.

Shoots co-cultivated with *P. arhizus* but not amended with tryptophan and control shoots with (Fig 4) and without NAA (Fig 3) all rooted at 20% (Table III). Co-cultivation with *P. arhizus* and with 0.3 μM NAA in the medium was the best rooting treatment (56.6%; Fig 2). Rooting with IAA was low (6.0%) and associated with extensive callus formation (Fig 5). Co-cultivation with *P. arhizus* improved rooting to 13.0% but rooting remained less than that of controls. However, callus production associated with the IAA treatment was greatly reduced in the presence of *P. arhizus* (Fig 5). No rooting was observed with a combination of NAA (0.3 μM) and IAA (0.5 μM). Although IAA has been detected in pure culture filtrates of *P. arhizus* (Frankenberger and Poth, 1987), auxin alone does not appear to be responsible for the mycorrhizal-enhanced root induction. Shoots cultured with *P. arhizus* in a tryptophan-amended medium produced the highest mean number of roots per shoot (3.7) as compared to controls (1.0) or to those cultured in unamended medium in the presence of *P. arhizus* (2.0). This is the first report of *in vitro* root induction on adventitious conifer shoots using *P. arhizus*.

Rooting percentages of micropropagated pine shoots were significantly increased when rooting occurred in the presence of *P. arhizus* in a NAA- or tryptophan-amended medium. Kim and Lee (1990) similarly observed improved rooting on cuttings from 20-yr-old *Quercus* trees when a 3000 ppm indole-3-butyric acid dip was coupled with *P. tinctorius* inoculation.

Both TLC and HPLC analysis of the broth extracts of NAA-amended cultures of *P. arhizus* revealed the presence of three acidic compounds which were not present in non-amended cultures of *P. arhizus*. One of these was identified as NAA (<10% recovery) by comparison with an authentic sample (TLC, HPLC and GC-EIMS). The other two acids appeared to result from NAA oxidation. GC-EIMS analysis of the methylated broth extracts indicated the presence of two different hydroxy derivatives of methyl NAA (M^+ *m/z* 200, and base peak *m/z* 141 $(M\text{-}CO_2Me)^+$) which had M^+ *m/z* 216 (35%) and a base peak *m/z* 157 $(M\text{-}CO_2Me)^+$ for the two unknown components. Work is in progress to determine the chemical structures of these two compounds.

Co-culturing micropropagated shoots with ectomycorrhizal fungi has been reported to result in mycorrhizae formation (Tonkin *et al.* 1989, Grellier *et al.* 1984).

Table I Comparative effects of 3 ectomycorrhizal fungi on rooting of de-rooted *Pinus monticola* seedlings.

Treatment	Total number of seedlings	Rooting (%) $P<0.005$	Mean number roots per seedling $P<0.05$	Total mean root length per seedling $P<0.005$
Control	19	78.9b*	1.7b	6.5b
Laccaria bicolor	20	100.0a	2.2b	9.2a
Rhizopogon rubescens	19	100.0a	2.5b	4.7b
Pisolithus arhizus	20	100.0a	2.8a	7.0ab

* Means within a vertical column followed by different letters are significantly different by contingency Chi-square for rooting or by Duncan's multiple range test for number of roots and root lengths.

Table II Percent rooting of micropropagated *Pinus monticola* shoots co-cultivated with *Pisolithus arhizus, Laccaria bicolor* or *Wilcoxina mikolae* on half-strength Gresshoff-Doy medium containing 1-naphthaleneacetic acid or tryptophan. Contingency χ^2's are also shown.

	*Pisolithus arhizus**	*Laccaria bicolor*	*Wilcoxina mikolae*	*Control*	*Mean*
Control	20.0	0.0	26.6	20.0	16.7a**
1-Napthaleneacetic acid 0.3 μM	53.3	26.6	0.6	20.0	26.7
Tryptophan 0.1 mM	46.6	13.3	13.3	13.3	21.7a
Tryptophan 0.01 mM	26.0	20.0	13.3	0.6	16.7a
Mean	36.7a	15.0b	15.0b	15.0b	

* Values represent mean values of 15 shoots per treatment.

** Means within a row or column followed by different letters are significantly different at $p<0.05$ by contingency χ^2.

Contingency χ^2

Comparison:	df	χ^2	P
media X rooting	3	2.539	0.4683
fungus X rooting	3	13.001	0.0046
media (fungus) X rooting	15	26.643	0.0317

Table III Rooting percentages of micropropagated *Pinus monticola* shoots co-cultivated with (+) or without (-) *Pisolithus arhizus* in the presence of tryptophan, indole-3-acetic acid (IAA) or 1-naphthaleneacetic acid (NAA) on half-strength Gresshoff Doy medium.

Medium amendment	*Piaolithus arhizus* (+/-)	Rooting (%) n = 15 $\chi^2 = 19.8350$ $p < 0.005$	Mean number of roots per shoot $p<0.001$
None	-	20.0bcd	1.0b
None	+	20.0bcd	2.0b
TRP (0.1 mM)	+	43.3ab	3.7a
NAA (0.3 μM)	-	20.0bcd	1.7b
NAA (0.3 μM)	+	56.6a	2.5b
IAA (0.5 μM)	-	6.0cd	3.0b
IAA (0.5 μM)	+	13.3cd	1.0b
NAA (0.3 μM) and IAA (0.5 μM)	-	0.0d	-

* Means within a vertical column followed by different letters are significantly different by contingency Chi-square for rooting or by Duncan's multiple range test for number of roots.

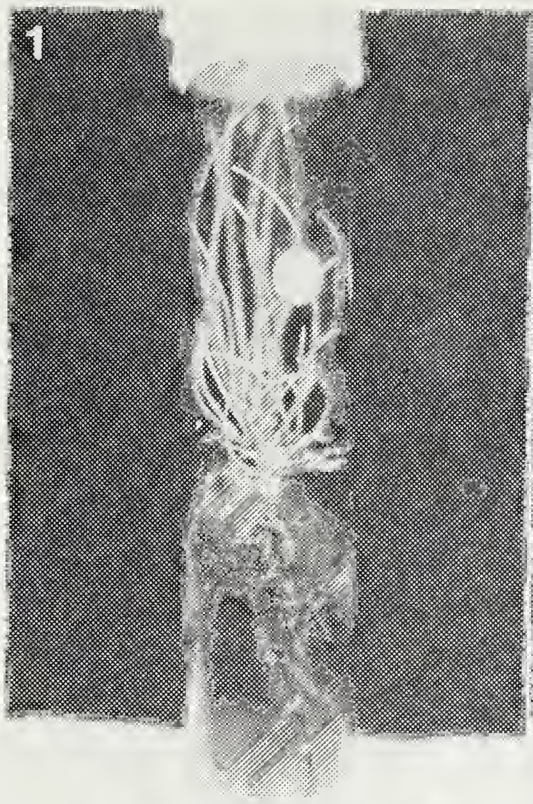
Fig 1 *Pinus monticola* shoot rooted in the presence of *P. arhizus* in Gresshoff Doy medium amended with tryptophan (0.1 μM). (Diameter of the tube = 25 mm)

Fig 2 *Pinus monticola* adventitious shoots rooted in the presence of *P.arhizus* in Gresshoff Doy medium amended with NAA (0.3 μM). (Diameter of the tube = 25 mm)

Fig 3 *Pinus monticola* control shoots rooted in Gresshoff Doy medium. (Diameter of the tube = 25 mm)

Fig 4 *Pinus monticola* shoots rooted in Gresshoff Doy medium amended with NAA (0.3 μM). (Diameter of the tube = 25 mm)

Fig 5 Adventitious *Pinus monticola* shoots on Gresshoff Doy medium amended with IAA (0.5 μM) (right) and in the same medium plus *P. arhizus* (left) showing the absence of callus tissue in the presence of *P. arhizus*. (Diameter of the tube = 25 mm)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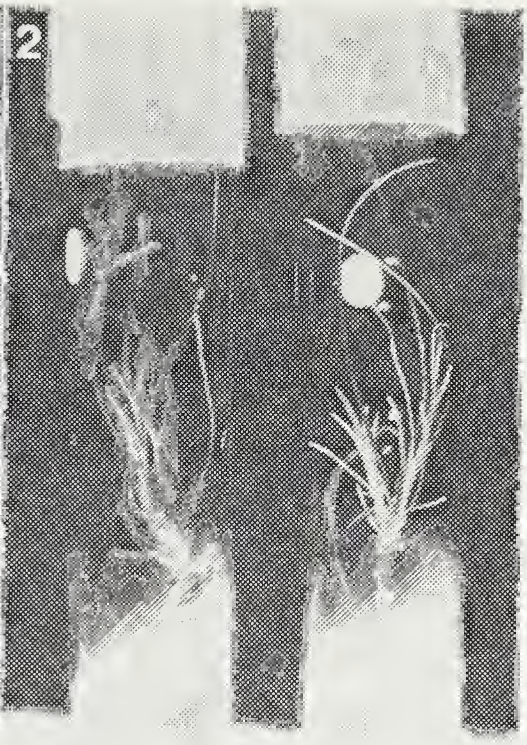
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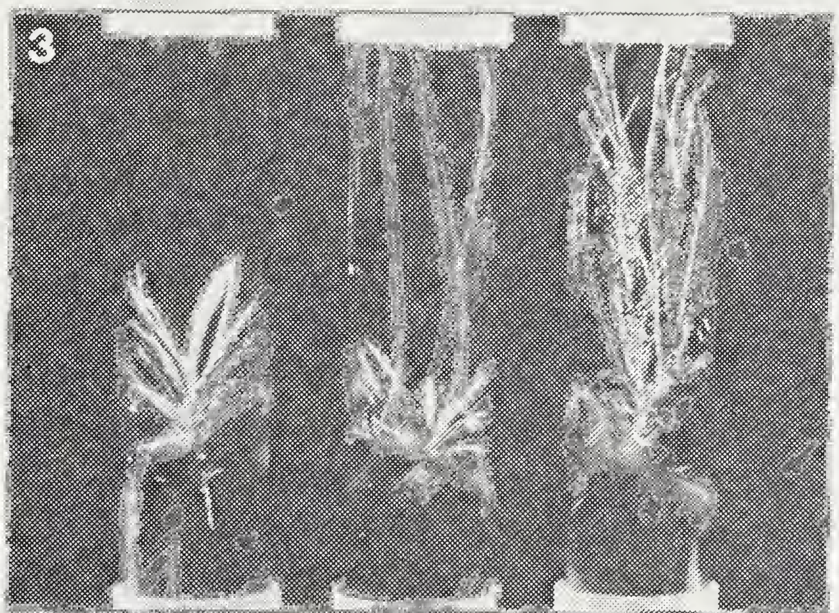
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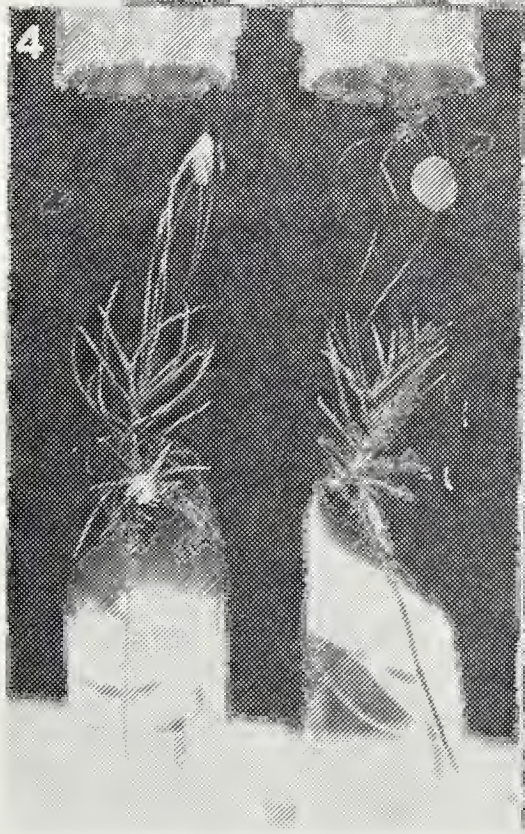
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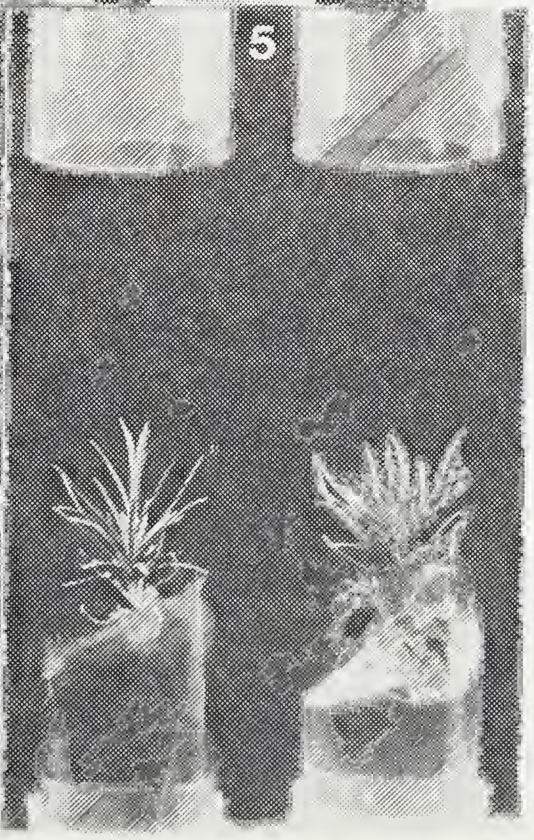
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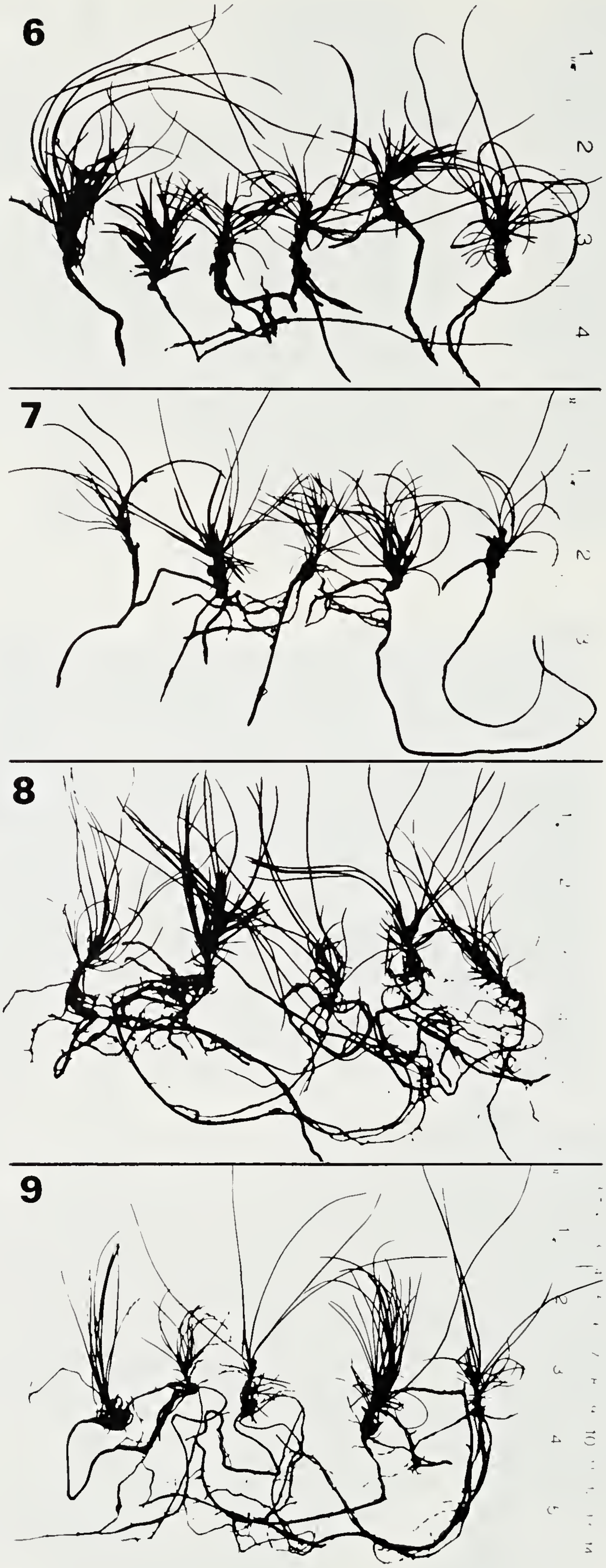

Figs 6-9 *Pinus monticola* adventitious shoots rooted in test tubes on Gresshoff Doy medium and transferred to a peat:vermiculite substrate in a greenhouse mistbed for 10 weeks: Fig 6 controls, Fig 7 NAA (0.3 μM) induced rooting, Fig 8 *Pisolithus arhizus* induced rooting, Fig 9 *P. arhizus* and NAA (0.3 μM) induced rooting.

However, Malajczuk and Hartney (1986) and Gay (1990) observed that ectomycorrhizae were initiated only on roots formed above the agar surface. The oxygen-poor agar substrate was probably detrimental to ectomycorrhizal formation in our experiment. Transferring these rooted shoots to a soil-like substrate *in vivo* did not result in ectomycorrhizal formation either. However, after two months in a greenhouse mistbed, the initial differences in root numbers per shoot, were still maintained (Figs 6-9).

Malajczuk and Hartney (1986) found that plantlets inoculated with ectomycorrhizal fungi survived transplantation from agar to peat substrate better than controls and Kim and Lee (1990) reported that the average survival rates of rooted *Quercus* cuttings were markedly improved when cuttings were inoculated with *P. tinctorius*. In liquid culture, *P. arhizus* produces extracellular metabolites which have been shown to inhibit a wide range of phytopathogenic fungi (Kope and Fortin 1990) and increased uptake of soil nutrients has been demonstrated with ectomycorrhizae (Harley and Smith 1983). Future studies should consider the effects of ectomycorrhizal inoculation treatments on root and shoot nutrition and the role of oxygenated NAA metabolites. Reinoculating with *P. arhizus* after root induction, or determining cultural conditions that are conducive to mycorhhizal formation as well as rooting may ultimately result in improved shoot as well as root quality and increased survival rates during transfer to field conditions, a critical stage in micropropagation.

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FIRST RECORD OF THE POTENTIAL NUISANCE ALGA *Codium fragile* ssp. *tomentosoides* (Chlorophyta, Caulerpales) IN ATLANTIC CANADA*†

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The green macroalga *Codium fragile* ssp *tomentosoides* was collected for the first time in Atlantic Canada at Graves Shoal, Mahone Bay, Lunenburg County, Nova Scotia, in December 1991. Casual observations in this area during the late 1980s suggest that it was present in 1989, possibly earlier. By late 1992, it had been found widely throughout Mahone Bay and eastward as far as Prospect Bay in Halifax County. The evidently rapid dispersal, large size of plants and abundant production of gametangia indicate that the semiconfined environment of bays on the Atlantic coast of Nova Scotia is favorable to the alga, which in consequence may be a serious foulant and competitor with the indigenous flora. It is probable that *C. fragile* ssp. *tomentosoides* was transported from New England to Nova Scotia either by recreational yacht traffic or in a mass of warmer water driven westward from the Gulf Stream.

La macro-algue verte *Codium fragile* ssp. *tomentosoides* a été collectionnée pour la première fois dans le Canada atlantique, c'est-à-dire, à Graves Shoals, Mahone Bay, comté de Lunenburg, Nouvelle-Ecosse en décembre 1991. Des observations d'occasion dans ce lieux pendant les dernières années '80 suggèrent sa présence en 1989 et même plus tôt. Vers la fin de l'année 1992 on l'avait trouvée largement répandue à Mahone Bay, et vers l'est aussi loin que Prospect Bay dans le comté d'Halifax. La dispersion manifestement rapide, la grande taille des plantes, et la production abondante de gamentagia indiquent que l'environnement enfermé à moitié des baies de la côte atlantique de la Nouvelle-Ecosse est propice à l'algue; celle-ci pourrait être une entité salissante importante et concurrencer avec la flore indigène. Il se peut que *C. fragile* ssp. *tomentosoides* ait été amené de la Nouvelle-Angleterre soit par des yachts récréationnels soit dans une masse d'eau plus chaud poussée vers l'ouest et provenant du Gulf-Stream.

Introduction

Codium fragile (Suringar) Hariot ssp. *tomentosoides* (Van Goor) Silva (Fig 1) is a widely distributed (Schneider & Searles, 1991) robust green alga of temperate to warm-temperate waters, considered to be endemic to the northwest Pacific (Silva, 1955). It was first found on the east coast of North America in 1957, at Orient Harbor on Long Island, New York (Carlton and Scanlon, 1985), following accidental introduction from western Europe, and has spread both northward and southward so that its known distribution in the northwest Atlantic in 1991 was from Bar Harbor, Maine (R. L. Vadas, pers comm.) to North Carolina (Schneider & Searles, 1991). Carlton and Scanlon (1985) have reviewed the dispersal of *C. fragile* ssp. *tomentosoides* along this coast, and considered various possible mechanisms of introduction and dissemination.

The spread of *C. fragile* ssp. *tomentosoides* in the northwest Atlantic has caused some concern as the species is a vigorous colonizer, rapidly forming dense stands (e.g.,

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[*] Dedicated to the memory of Kenneth A. Harrison

[†] NRCC 34838

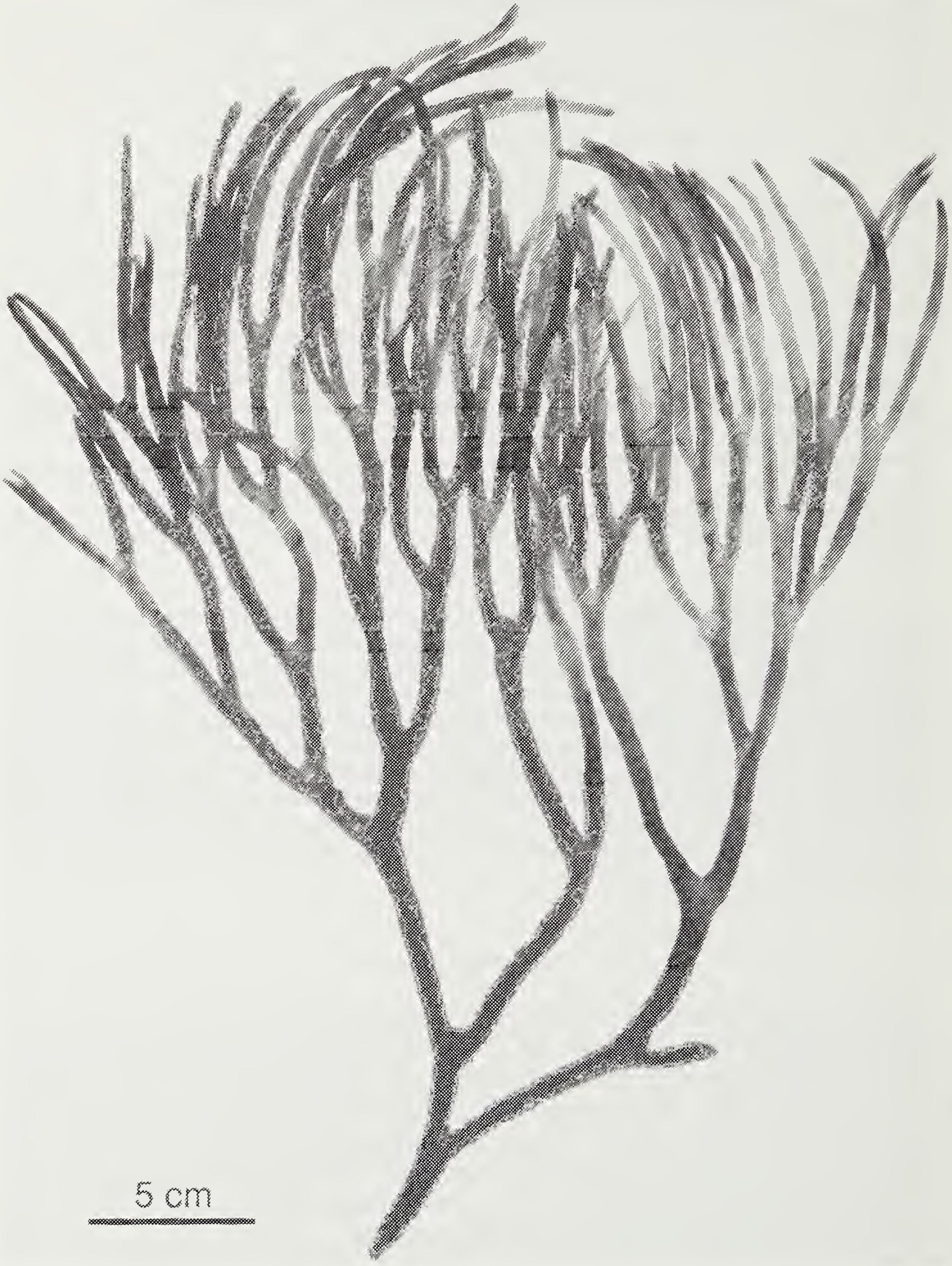

Fig 1. *Codium fragile* ssp. *tomentosoides*. Portion of a large plant. The spongy, repeatedly forked, cylindrical to compressed fronds are unmistakable in the seaweed flora of eastern Canada.

Ramus, 1971; Prince, 1988) in competition with the indigenous flora. Where abundant, it also has been a nuisance to fishing activities, overgrowing valuable shellfish beds and clogging dragnets (Fralick & Mathieson, 1972; Carlton & Scanlon, 1985). In consequence, much attention has been directed to determining factors affecting its growth, reproduction and dispersal in American waters (reviewed by Carlton and Scanlon, 1985).

The northward progress of *C. fragile* ssp. *tomentosoides* through Maine, however, has been relatively slow and populations are disjunct, restricted to bays where water temperatures are warmer than on outer coasts. This has suggested that the species is nearing the northern limit of its distribution in the western Atlantic, and that the cold waters of the Bay of Fundy approaches could prevent its direct passage to Nova Scotia.

We now report on the arrival of *C. fragile* ssp. *tomentosoides* in Nova Scotia, suggest means of introduction, and, in view of the nuisance reputation of this alga, discuss implications of its presence for the local marine flora and fauna.

Observations

The first collection was made on 1 December 1991, from scallop aquaculture floats at Graves Shoal (44°33′N, 64°12′W) in Mahone Bay (Fig 2). Plants grew densely among mussels 2-4 m below mean low water, and were up to 45 cm long, with gametangia. Specimens were identified as *C. fragile* ssp. *tomentosoides* by the criteria of Silva (1955) and Burrows (1991), and are conserved in the herbaria of the National Research Council, in Halifax (NRCC, No. 10883). Plants had also been noted during 1989 and 1990, but were not collected. Up to late 1991, the species appeared to be confined to the floats, as no specimens were observed attached to natural substrate on Graves Shoal and none was seen in the drift on nearby beaches.

At about the same time, *C. fragile* ssp. *tomentosoides* was found, attached to mussels, on Little Tancook Island (44°28′N, 64°08′W) near the mouth of Mahone Bay. A specimen was submitted for identification to G. J. Sharp (Fisheries and Oceans Canada), who gave it to the Institute for Marine Biosciences (NRCC 10889).

The following spring and summer, incidental observations were made at various sites in Mahone Bay and adjacent shores. At about this time, the large thalli disappeared from the aquaculture system at Graves Shoal and were replaced by a dense growth of younger fronds. Plants of *C. fragile* ssp. *tomentosoides* appeared on several occasions adrift near the head of the bay, and attached thalli were found on several reefs (Fig 2), including Graves Shoal, usually at 1.5-5 m depth on rocky substrate in areas of moderate exposure. Owing to their large size (to 55 cm long), plants were a conspicuous, sometimes subdominant, element of the flora, and on Sheep Ledge (44°30′N, 64°16′W) were estimated to constitute as much as 40% of the cover. Larger thalli had a tendency to detach and accumulate in depressions at maximum depths of about 12 m, forming deposits up to l m thick but apparently remaining in healthy condition, at least near the surface of the mass. In these situations, they sometimes verged on beds of Laminaria, presumably to the detriment of understory flora and fauna. The easternmost location of the species observed thus far was at Kelly Ledge (44°29′N, 63°46′W), near the mouth of Prospect Bay, Halifax County (Fig 2). Specimens, which were not collected routinely, are deposited in NRCC and at Acadia University (ACAD).

The alga was not observed in sites with a predominance of sand or cobble substratum [Frog Island (44°31′N, 64°17′W); Round Island Nubble (44°29′N, 64°16′W); Little

Duck Island (44°22′N, 64°11′W), A. Windust, Dalhousie University, pers. comm.], although some of these sites were close to flourishing populations.

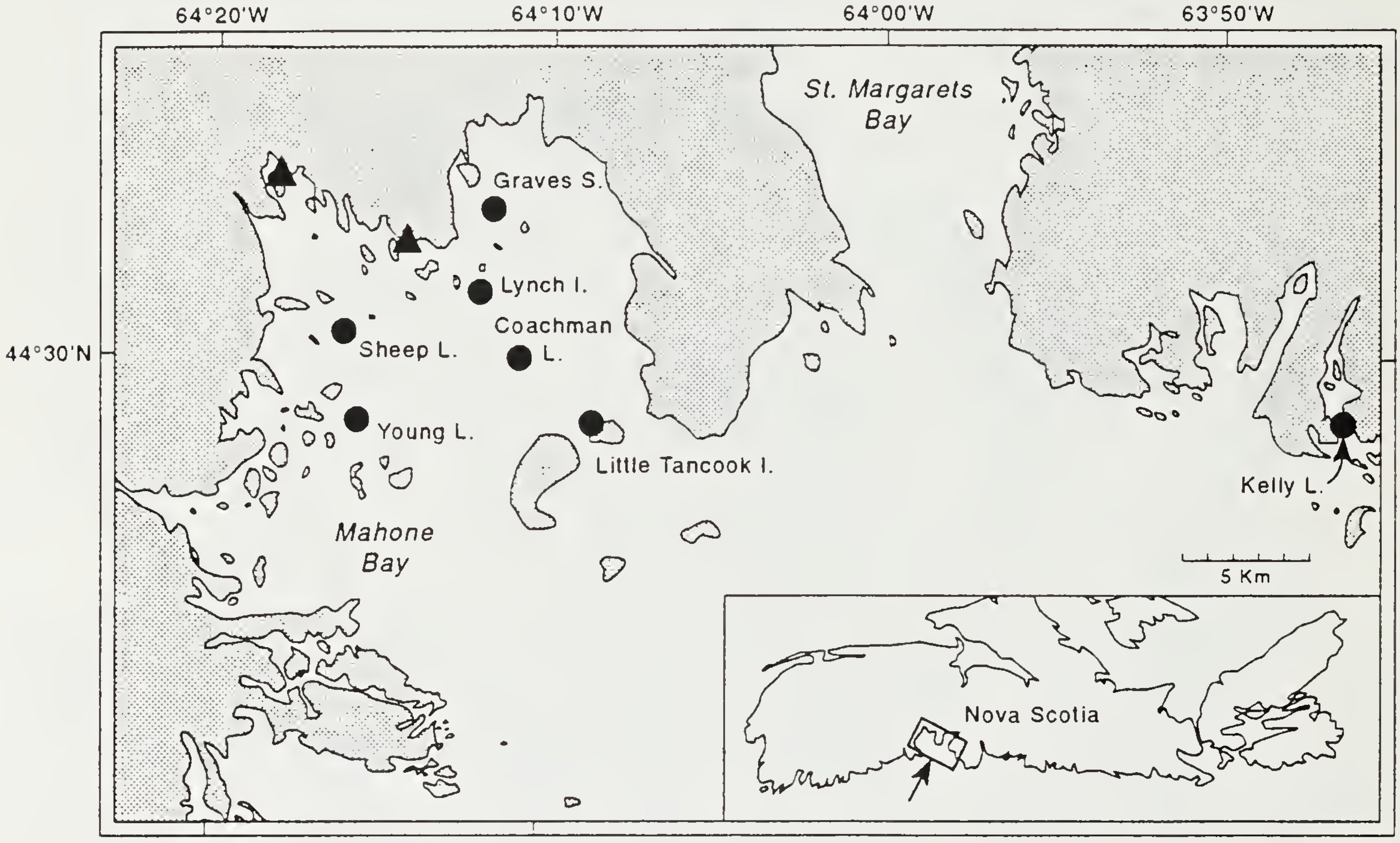

Fig 2. Map of Mahone Bay, Nova Scotia and adjacent coast eastward to Prospect Bay, showing locations of *Codium fragile* ssp. *tomentosoides* recorded up to autumn 1992. ●-attached plants; ▲-drift specimens only. Inset: map of Nova Scotia, showing the area enlarged (arrow).

Discussion

Means of introduction. The means by which a species is introduced into a new region is often a matter of speculation. *Codium fragile* ssp. *tomentosoides* is considered to have been introduced from Europe to New England as a foulant on ship's hulls (Carlton and Scanlon, 1985), although introduction as an epibiont on oysters imported for aquaculture remains a distinct possibility. Given the volume of boat traffic between New England and Nova Scotia, and between Europe and eastern North America, multiple introduction of *C. fragile* ssp. *tomentosoides* should be considered possible. The alga is able to settle on hulls imperfectly coated with antifouling paint, to colonize substrata with minimal surface relief, to survive shear stresses as a basal holdfast, and to tolerate large changes in temperature and salinity (Carlton and Scanlon, 1985). However, introduction via commercial ships is often restricted by the absence of *C. fragile* ssp. *tomentosoides* in harbours, perhaps a result of pollution, and by the lack of opportunity to colonize hulls owing to rapid turnaround time. Although the species has been present in Bar Harbor since the late 1970s or early 1980s (R. L. Vadas, University of Maine, pers. comm.), it evidently has not been transported to Yarmouth, Nova Scotia, by the regular ferry because it has not been seen in recent surveys of the algal flora (G. J. Sharp, Fisheries and Oceans Canada, pers. comm.). Smaller craft, for example yachts arriving in Nova Scotia from various bays along the eastern American seaboard, are a possible means of introduction, although attachment of *C. fragile* ssp. *tomentosoides* to such vessels would require an uncharacteristic neglect of the hull. The ability of propagules of *C. fragile* ssp. *tomentosoides* to survive passage in ballast or bilge water has not been investigated.

Relocation of shellfish bearing primordia or residual holdfasts of *Codium* to new culture sites is thought to be a major mechanism of dispersal along the American coast (Carlton and Scanlon, 1985). However, the mussel and scallop stocks currently being cultivated in Mahone Bay are of domestic origin (C. T. Enright, N. S. Dept. of Fisheries, pers. comm.), and the history of the aquaculture floats from which the first specimens were collected precludes their being the vector for introduction. Despite the sanitation and quarantine requirements for live shellfish imported across international boundaries, it is possible that *C. fragile* ssp. *tomentosoides* was brought to Nova Scotia by casual importation of contaminated shellfish.

Importation of *Codium* as a packing material for live fishery products such as lobsters and baitworms has been proposed as a means of dispersal (Carlton and Scanlon, 1985), and we have no information on the incidence of this usage for fishery products being imported into Nova Scotia. However, there are few if any facilities in Mahone Bay for reception of such products, and movement of fishery products in this area is largely or wholly in the opposite direction. *Codium fragile* ssp. *tomentosoides* arriving this way is more likely to have been discarded from the provisions of a private vessel.

A final potential mechanism for introduction is the occasional westward movement of eddies from the Gulf Stream to the Nova Scotian coast. These masses of warm water, which have been arriving more-or-less regularly since at least the early 1900s (J. Gilhen, Nova Scotia Museum, pers. comm.), contain more southerly species (e.g., Markle et al., 1980; Gilhen and Coad, 1991), and if they penetrate coastal waters to a suitably warm environment, can effect introductions. Water temperatures along this coast were frequently about 2°C above normal during the mid-1980s (R. H. Loucks Oceanology Ltd. et al., 1991) and, in outer St. Margarets Bay, near-surface maximum temperatures in 1990 were 19-21°C (Gregory et al., 1991), well above the average maximum of 14.3 ± 3.2°C for the general area (R. H. Loucks Oceanology Ltd. et al., 1991). Such incursions of warmer water may well have brought *C. fragile* ssp. *tomentosoides* to our shores.

Codium *in the Nova Scotian environment.* It is evident that *C. fragile* ssp. *tomentosoides* has become permanently established in Nova Scotia, and has probably been here since the late 1980s. The implications for the resident flora or fauna in habitats preferred by the alga are as yet undetermined. In New England, it is classified as "weedy" (Kingsbury, 1969), "invasive" (Prince and LeBlanc, 1992), and "a serious pest" (Gosner, 1978), and has rapidly colonized rocky areas stripped of kelp by sea urchins, to form dense stands (Prince, 1987, 1988). However, there is little evidence of wholesale permanent displacement of other algae from such habitats. To the contrary, the population of *C. fragile* ssp. *tomentosoides* at Boothbay Harbor, Maine apparently has declined in abundance from levels noted shortly after invasion (Fralick and Mathieson, 1972; Prince, 1988). In addition, recent studies on sea urchin herbivory (Prince, 1991; Prince and LeBlanc, 1992) have shown that *C. fragile* ssp. *tomentosoides* is itself readily grazed by green sea urchins, and this may help to keep populations in check.

Temperature is the primary factor affecting growth and reproduction of *C. fragile* ssp. *tomentosoides* (Fralick and Mathieson, 1973). The reported minimal temperatures for vegetative growth are 10° to 13°C, and for formation of gametangia are 12° to 15°C

(Carlton and Scanlon, 1985, and references therein), and these must be sustained for sufficient lengths of time in order for the species to become established. It is evident from the slow northward progress of the sub-species through the Gulf of Maine, its restriction there to the warmer waters of bays (Carlton and Scanlon, 1985), and its reduced growth rates compared to more southern localities (Fralick & Mathieson, 1973) that the alga is approaching the northernmost limit of its distribution in eastern North America. On the other hand, if it penetrates the southern Gulf of St. Lawrence where summer surface water temperatures are relatively high, it may become more widespread unless the protracted ice cover and low temperatures of winter in this area discourage its survival. However, Prince (1988) noted that *C. fragile* ssp. *tomentosoides* at Appledore Island, Maine was flourishing in a thermal regime considered suboptimal for the species, and the increased incidence of fragmentation during colder months (Fralick and Mathieson, 1972) could in fact encourage the spread of the alga in such situations, via dispersion of gametangial thalli. Comparison of northern and southern populations has shown that selection for the different thermal regimes has occurred, with the formation of cold-water ecotypes in Maine (K. C. Malinowski, in Carlton and Scanlon, 1985). Notwithstanding this thermal plasticity, temperatures in Mahone Bay satisfy the requirements of more southerly populations, being suitable from early May to late October for vegetative growth and from mid-May to late September for formation of gametangia.

Reproduction of *C. fragile* ssp. *tomentosoides* occurs via monoecious gametangia containing anisogametes (Prince, 1988) and by vegetative propagation. We have noted abundant gametangia on plants collected from August to December (cf. Burrows, 1991), although we do not know whether they were uniformly functional during this period. The regenerative capacity of this alga is a significant factor in the success of the species. The cushion-like base can regenerate erect fronds detached by turbulence, and the regrowth of small thalli on the aquaculture apparatus from which the original collections were made was probably due to regeneration from basal residua. In addition, detached fronds are capable of reattaching themselves to solid substrate via their basal filaments, although the reattachment process is much retarded by the lower temperatures that foster fragmentation (Fralick and Mathieson, 1972).

As the environment inhabited by *C. fragile* ssp. *tomentosoides* is also used for marine aquaculture, we expect that one of the most significant adverse effects of this species will be as a foulant on aquacultural apparatus. The species is notable for its ability to colonize vacant substrate indiscriminately (Carlton and Scanlon, 1985). Rapid and luxuriant growth of thalli can severely clog mesh containers and impede water circulation. Once established, plants are difficult to eliminate as their spongy texture resists desiccation, and residual holdfast filaments will readily regenerate. To retard the spread of plants to other areas, measures should be taken to ensure that culture apparatus and shellfish are free of this species before relocation to new sites.

Acknowledgements

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UNDESCRIBED MAMMOTH *(Mammuthus)* TEETH FROM GEORGES BANK AND NOVA SCOTIA

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Ten molar teeth of mammoths are described, nine of them dredged by fishermen from the northeastern corner of Georges Bank while the tenth was in gravel from near James River, Nova Scotia, although found at St. Francis Xavier University. Measurements and morphological features are discussed and the molars are referred to woolly mammoth *(Mammuthus primigenius)* although a few specimens are lower crowned than is usual and may represent a local variant.

On décrit dix dents molaires de mammouths, dont neuf ont été dragués par des pêcheurs au coin nord-est du Banc Georges tandis que le dixième a été trouvé dans du gravier près de James River, Nouvelle-Ecosse et a été découvert plus tard a l'Université St. Francois Xavier. On discute des mesures et des traits morphologiques, et ces molaires ont été placées dans le groupe du mammouth laineux (*Mammuthus primigenius*), bien que quelques spécimens démontrent des couronnes moins élevées que l'ordinaire, et représentent peut-être une variante locale.

Introduction

In 1967, Whitmore *et al.* discussed the occurrence of elephant teeth on the Atlantic continental shelf, including four mastodon and three mammoth teeth from Georges Bank; two of these specimens were illustrated in that paper (Fig 1.1,1.2). Nova Scotia scallop fishermen operate mainly on the northeastern edge of Georges Bank and it appears that fossils of various types are found particularly near an elongate bar running SE from 42°00'N 67°30'W to 41°53'N 67°20'W, known as "The Rip". Ten more specimens have come to our attention and probably many more are in private hands. Details of the discoveries are often scanty or lacking but the available background data are set out in Table I.

Description

There are two mammoth teeth in the collections of the Nova Scotia Museum (#1 and #2 in Table I). Specimen #1 is the front half of the left lower molar, possibly M_1 or M_2. There are 8 lamellae preserved, all in wear, and the last plate is somewhat shattered. The whole specimen has been rolled and abraded as if it had been part of a gravel; the roots are completely gone. The enamel borders of the plates are generally parallel and the enamel is thin and crimped. Measurements are given in Table II.

Specimen #2 was collected by Eugene d'Entremont in 1963 from Georges Bank in his ship "Acadian Pal". It is the back half of an upper molar, probably RM^3 (Fig 1 A,B). There are 9 plates preserved as well as a terminal half plate, which is broken. The front

Table I Some data on mammoth teeth from Georges Bank and Nova Scotia

No.	Collection	Position	Year	Collector/Vessel
1.	NSM 984 GF 10.1	Unknown	Unknown	
2.	NSM 984 GF 23.1	41°55′N, 67°30′W	1963	Eugene d'Entremont/ "Acadian Pal"
3.	NMC 47728	Georges Bank	1967	Unknown (Dr. L.P.Chiasson)
4.	Sollows "A"	c42°00′N, 67°30′W	c1970	Unknown (J.D.Sollows)
5.	Milton School Yarmouth "B"	ditto	c1970	Unknown (J.D.Sollows)
6.	YCM "C"	ditto	c1970	Unknown (J.D.Sollows)
7.	Huntsman Aquarium	41°43.5′N, 67°9.3′W	1977	/"Sharon and Dawn II"
8.	James River	James River, N.S. gravel pit	1980	Bishop Malcolm MacEachern
9.	Jeddry	41°20.4′N, 66°20.1′W	1981	Victor Jeddry
10.	YCM NS 92.6	Georges Bank	1985	Blair Doucet /"Lady Denise"

NSM = Nova Scotia Museum, Halifax; NMC = Canadian Museum of Nature, Ottawa;
YCM = Yarmouth County Museum, Yarmouth; c = approximately.

three plates are in full wear and the remainder lightly worn to show small enamel islands representing 5 digitations in the next three plates, 4 in the last two. The enamel is thin and crimped. Measurements are given in Table II.

In the mid-1960s, members of the Biological Research Station at St. Andrews, New Brunswick, were examining strange catches (including a Miocene shark tooth, an unidentified crab, and a *Perna* shell) brought in by Georges Bank scallop fishermen. They also received a fine but fragile mammoth molar (#3) from Dr L.P. Chiasson of the Biology Department at St. Francis Xavier University, Antigonish; this specimen is now in the Canadian Museum of Nature in Ottawa (NMC 47728). The molar had been impregnated with beeswax to preserve it, and may be the most complete upper third molar of a mammoth to come from the Atlantic continental shelf. It has 18 plates preserved but with one or two broken away at the talon; the 4th plate is badly shattered and part was lost. Six of the plates are in wear. The unworn plates show 5-8 digitations and the worn anterior plates produce rather narrow irregular islands, but plates #2 and #3 show three separate islands — a median and two laterals. The enamel is thin and finely crimped. The greatest length perpendicular to the plates is 215 mm and when complete the tooth may have measured 225-230 mm. The greatest breadth of 81 mm is at the 8th plate, while the maximum height is 155 mm; this gives a hypsodonty index

Table II Measurements on mammoth teeth from Georges Bank and Nova Scotia

No.	Collection	Tooth	P	L	W mm	H mm	Et mm	LF	100 H/W
1.	NSM 984 GF 10.1	LM$_2$?	+8+	87	54.9	81	1.3-1.5	9	158
2.	NSM 984 GF 23.1	RM^3	+9x	127.5	89	104	1.4-1.7	8	117
3.	NMC 47728	RM^3	18+	215+	81	155	1.5-1.9	8.5	191
4.	Sollows "A"	RM$_2$	+14	186+	69	80+	1.5-2.0	7.5	116+
5.	Milton School Yarmouth "B"	LM$_2$	+14	145+	68	56+ (90e)	1.5-2.0	10	132e
6.	YCM "C"	RM$_3$	+18x	206+	61.5	73+ (95e)	1.5-1.8	9	154e
7.	Huntsman Aquarium	RM$_3$	x17x	185	61.5	104	1.6-2.1	10	169
8.	James River	RM^2	+10	143+	57	76+	2.0	8	133+
9.	Jeddry	RM$_3$	+16+	271+	56.5	79+	1.5-1.9	6.5	140+
10.	YCM NS 92.6	LM^3	+15x	201e	77.5	94+ (110e)	1.5-2.0	7.5	121+ (142e)

P = number of plates preserved; L = length normal to plates; W = width of widest lamella; H = height of tallest plate; Et = enamel thickness; LF = lamellar frequency (plates per dm); 100 H/W = hypsodonty index; + = incomplete (before P, anterior lost, after P posterior lost); x = terminal partial plate; e = estimated

(H x 100/W) of 191, which is greater than that of any other tooth from the shelf described by Whitmore *et al.* (1967) or those described here (Table II). It is thus more like the typical *Mammuthus primigenius* than many of the other specimens from the continental shelf. However, one of the best preserved specimens (USNM 23569) dredged up off Cape Henry, Virginia (36°51.5′N, 75°02′W) and reported by Whitmore et al. (1967) was later referred to a woolly mammoth (Harington and Ashworth, 1986).

One of the authors (JDS) lived in Yarmouth and, while a student at Dalhousie University, assembled material available locally and collected near The Rip, which he described for a class assignment. The collection included some derived Cretaceous or older specimens, numbers of Miocene and Pleistocene molluscs, teeth of the Miocene shark *Carcharodon,* walrus tusks and some elephant remains. He reported that in 1966 an 8-foot long rib was dredged up but broke into several pieces en route from Georges Bank to Yarmouth. There was one well-preserved tooth of an American mastodon, *Mammut americanum,* 165 mm in length and 89 mm in width, bearing five pairs of cusps. Three mammoths were represented, all by lower molars. Of those three, Specimen "A" (#4) is a damaged RM$_2$ in full wear with 14 ridge plates preserved, part of the front probably already lost through natural wear but the hindmost plates are still in an early stage of attrition. The tooth was largely covered by a film of bituminous material, possibly bunker oil. Specimen "B" (#5) is now in the collections of Milton

Elementary School in Yarmouth (Fig 1 C,D). The tooth is probably an LM_2, also with 14+ ridge plates but the crown is more worn than "A" and had already lost part of the anterior plates through natural wear of the crown. The original height was probably close to 90 mm. The anterior four plates are broken up into residual elongate enamel islands. Specimen "C" (#6) is now in the Yarmouth County Museum. It is an RM_3 with 18+ ridge plates, the talon being broken and at least 2 plates lost. The crown is widest at the 9th lamella and tapers towards the back to one quarter of the width of lamella #9 (Fig 1 E,F). The enamel is moderately wrinkled in the anterior half of the crown but less so in the back half. Plate #13 shows signs of separation into two major enamel islands, plates #14, 15, 16 and 18 exhibit 4 islets but plate #17 has five rounded digitations. In all these specimens the plates are compressed and parallel sided, although #2 shows some tendency for a median expansion in the more worn lamellae. The original height of the crown was estimated to be close to 95 mm. Dimensions are shown in Table II.

An excellent RM_3 (#7) is on display at the Huntsman Aquarium in St. Andrews, New Brunswick. It was found by the scallop dragger "Sharon and Dawn II" in July 1977 at a depth of approxiately 35 fathoms (70 m) at the position shown in Table I. It was broken in transit but has been beautifully repaired. There is a small anterior "half" plate, 17 complete lamellae and a small talon, but nothing appears to have been lost anteriorly (Fig 2 A, B). The first 9 plates are in full wear across the crown, plates #10-13 show a central enamel island separated from the lateral islands but the last four plates show the 4 or 5 digitations that merge with wear into the central enamel ring. The enamel is crimped. The height of the unworn 14th plate is 104.3 mm and the maximum width of 61.5 mm is at the 6th plate, giving a hypsodonty index of 169 which is greater than that of any of the other lower molars from the shelf. There are 10 plates to a 100 mm length and these measurements and the morphology are well within the range of variation for the woolly mammoth, *Mammuthus primigenius*.

Specimen #8 was not recovered from the continental shelf but was found in 1980 by the late Bishop Malcolm MacEachern on the playing fields of St. Francis Xavier University, Antigonish, in gravel fill brought from a pit near James River, Nova Scotia. A mastodon molar was also found in this gravel. Although mastodon remains have been discovered at several sites in Nova Scotia, this is the only mammoth tooth to have been recovered on land. Investigation by Professor W.S Shaw of the Department of Geology at St Francis Xavier University suggested that the teeth were indeed brought in from gravel pits near James River and were not discards from some fisherman's collection. As might be expected, specimen #8 is somewhat damaged, especially at the side of the plates, and part of the front has been lost. According to the degree of freshness of the breaks, the sides and tips of the roots were broken off early, and fragments from the anterior and posterior were lost more recently, perhaps in transport and deposition at the site. The tooth is an upper molar (probably RM^2), in moderately advanced wear. Ten of the plates remain but several have been lost from the front through natural attrition and damage. Dimensions are given in Table II and the specimen is shown in Fig 2 C,D.

Another mammoth tooth (#9) was brought up in a scallop dredge on Georges Bank in July 1981 by Victor Jeddry of Meteghan, Nova Scotia, and is in his possession. It is an RM_3 with 16+ plates preserved (#9), and the lamellar frequency is only 6.5 plates in 100 mm. The talon is lost and it is also possible that one or more plates had been shed anteriorly. The tooth belonged to a relatively old individual as the anterior three plates were worn to a tract of dentine with residual scraps of enamel on the right (buccal) side. The last two preserved plates show only rings of enamel, not yet united

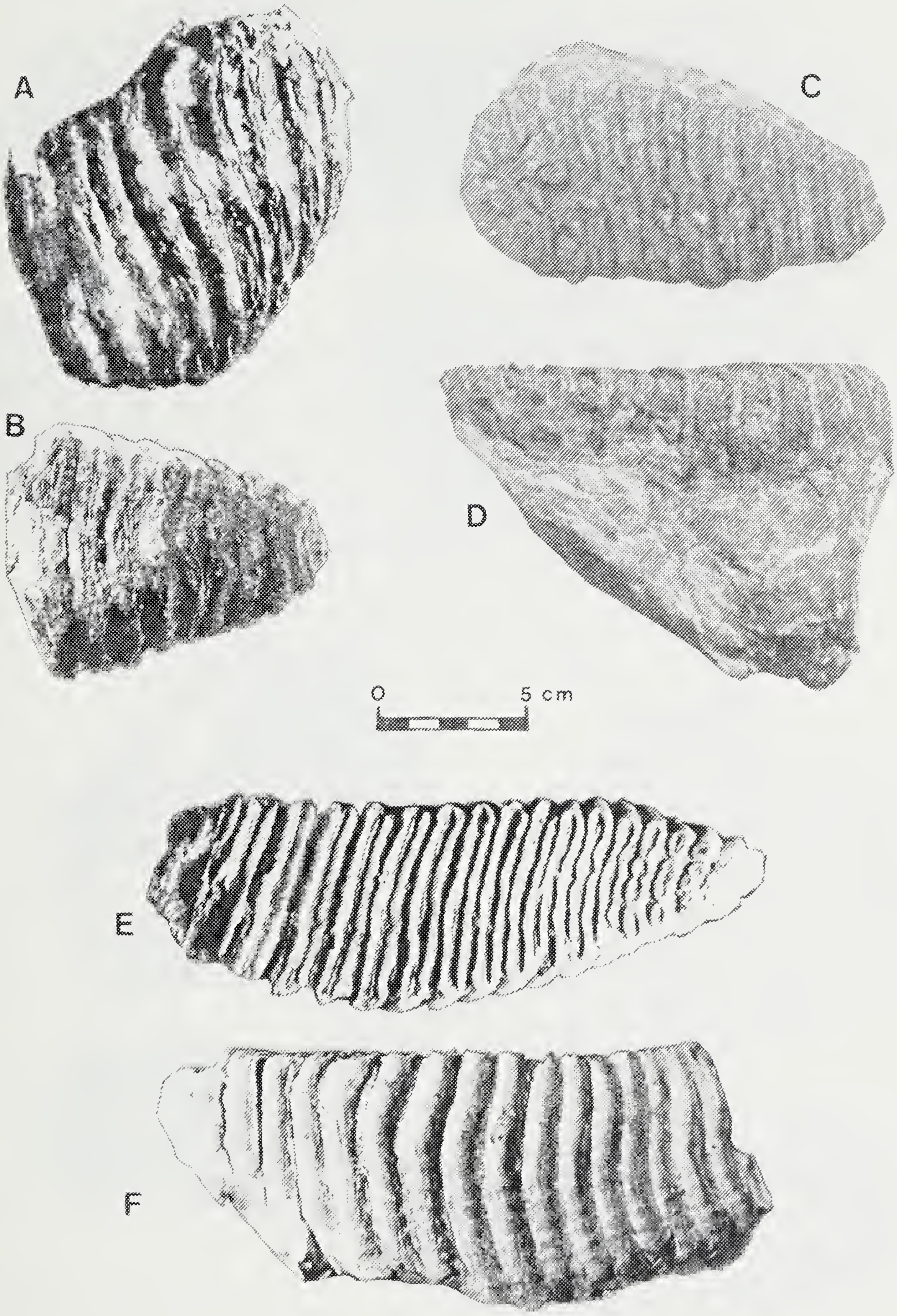

Fig 1 A, B: Back half of right upper third molar, NSM 984 GF 23.1 (#2), in the Nova Scotia Museum collections, seen in inner lateral and occlusal views. C, D: Well worn left lower second molar (Milton School "B"; #5) seen in occlusal and outer lateral views. E, F: Right lower third molar (YCM "C"; #6) seen in occlusal and inner lateral views. One third natural size.

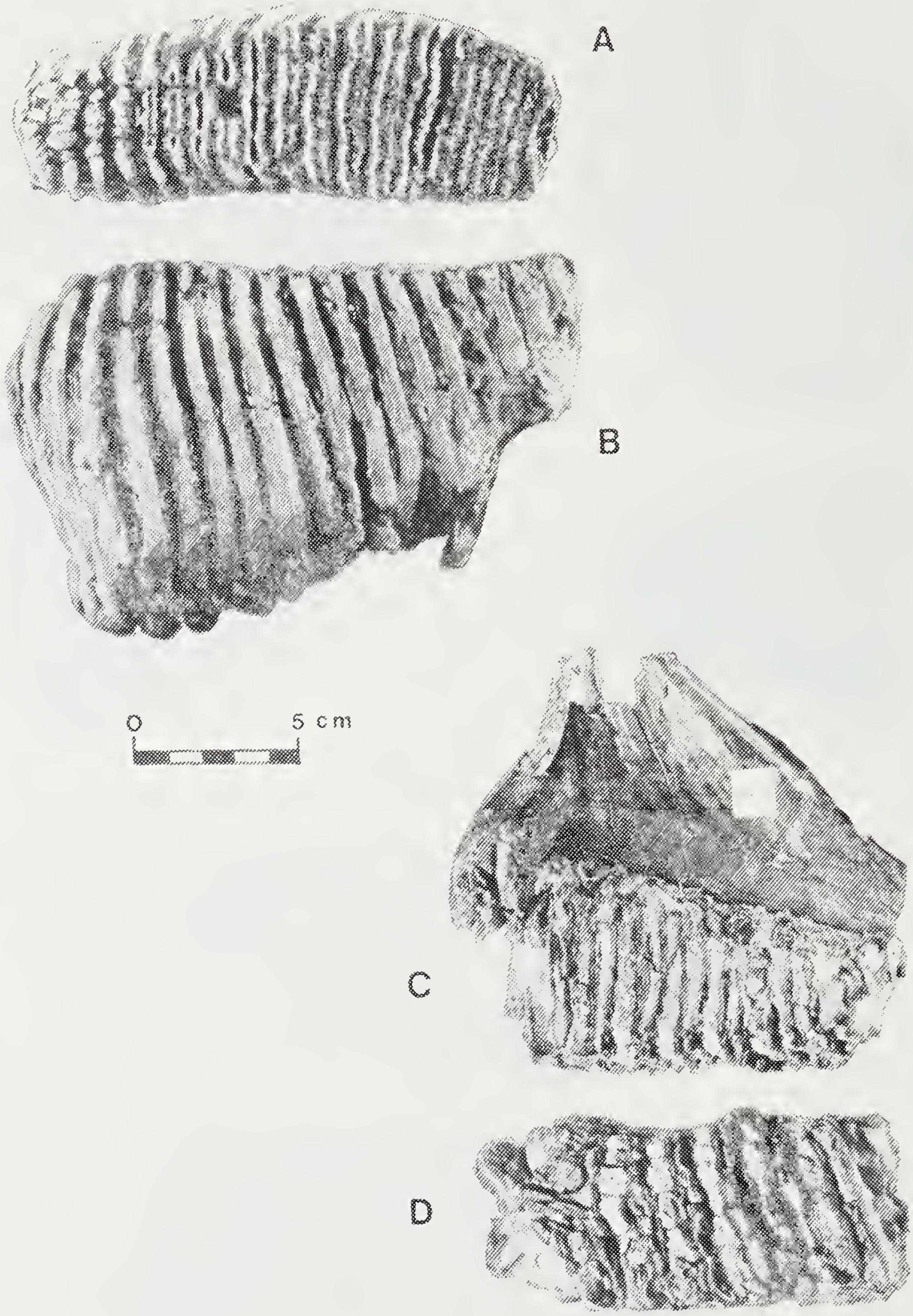

Fig 2 A, B: Complete right lower third molar in the Huntsman Aquarium collections (#7), seen in occlusal and outer lateral views. C, D: Right upper second molar (#8) found at St Francis Xavier University, Antigonish, in gravel brought from James River, Nova Scotia, seen in outer lateral and occlusal views. This is, so far, the only woolly mammoth tooth found on the Nova Scotian mainland. One third natural size.

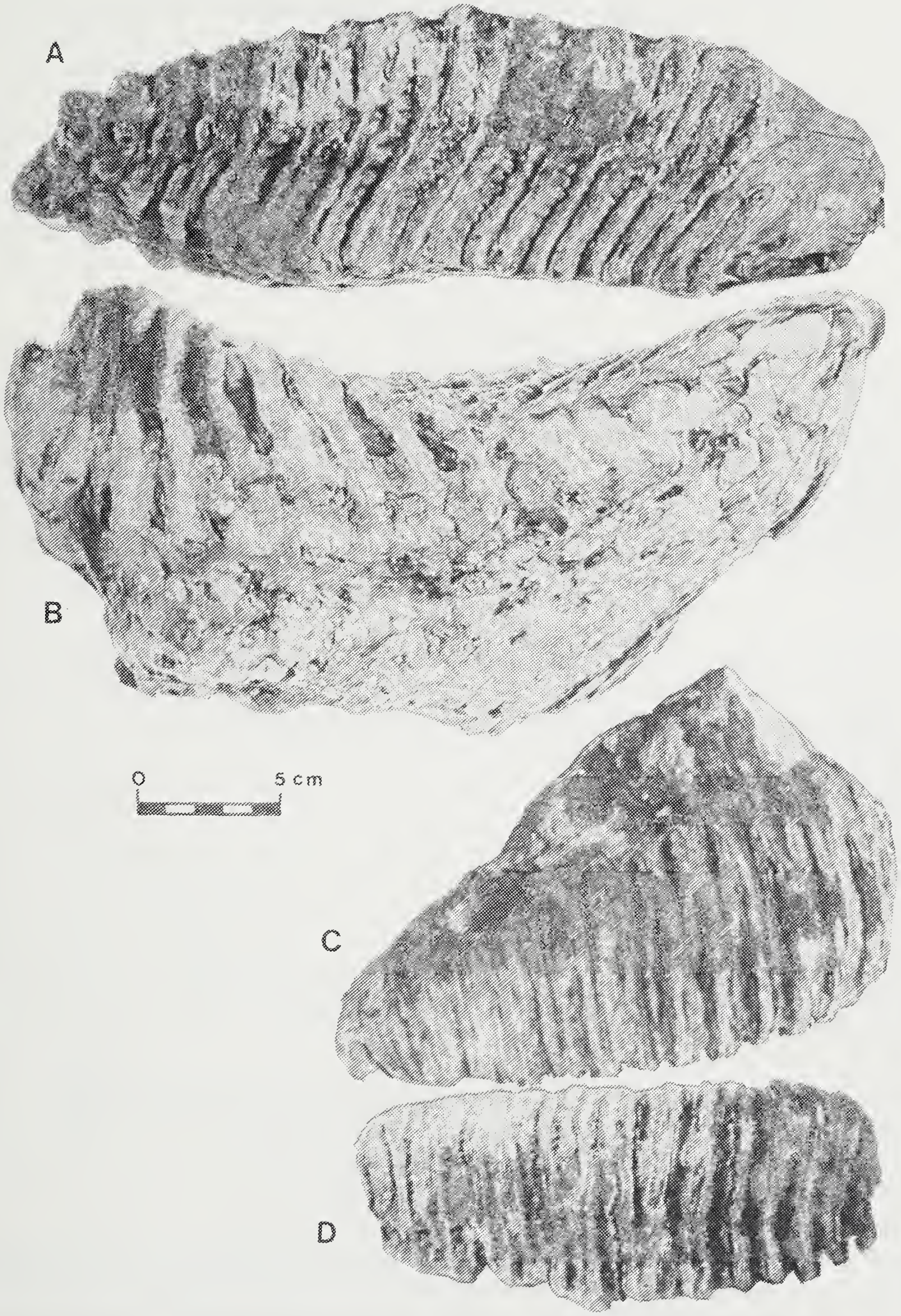

Fig 3 A, B: Left upper third molar, YCM NS 92.6 (#10), in the Yarmouth County Museum collections, seen in outer lateral and occlusal views. C, D: Right lower third molar, found by Victor Jeddry (#9) seen in outer lateral and occlusal views. Note abnormal asymmetrical concave wear surface. One third natural size.

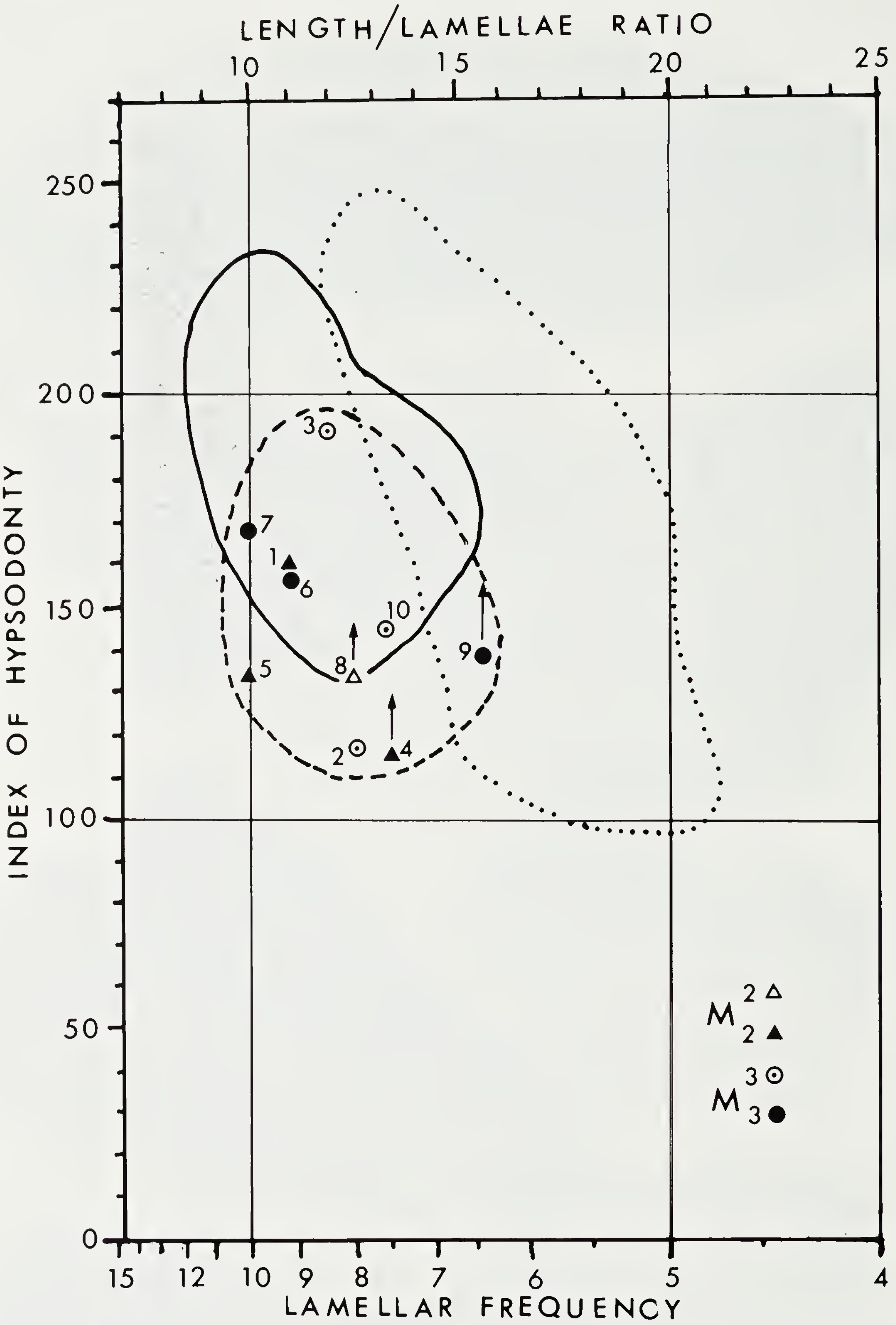

Fig 4 Diagram showing the relationship between the hypsodonty index and the lamellar frequency for the ten teeth described in this paper. The dashed line encloses the plots for the ten specimens considered here. The solid line shows the observed range of variation for upper and lower second and third molars of Holarctic *Mammuthus primigenius* (mainly from data in Maglio, 1973) while the dotted line encloses the range of variation found in North American *Mammuthus columbi* (after Whitmore *et al.* 1967). The arrows above #4, #8 and #9 indicate that the hypsodonty index is a minimum value and was probably greater.

into the characteristic narrow islands. The wear is abnormal, for the left (lingual) side was hollowed below the usual planar surface and the surface also cuts obliquely across the lamellae (Fig 3 A,B). The enamel is crimped. The dimensions are given in Table II.

The tenth specimen to be included here was found by Mr. Blair Doucet of Yarmouth about 1985 on the scallop dragger "Lady Denise", and is now in the Yarmouth County Museum (YCM NS 92.6). It is a fairly heavily worn LM^3 with part of the root system, of which the anterior portion has been broken away recently. Fifteen plates remain but two or three additional anterior plates were present originally, and the posterior terminal plate is lost (Fig 3 C,D). Measurements are given in Table II.

Discussion

The mammoth teeth available to Whitmore *et al.* (1967) resembled those of the woolly mammoth in the multiplicity of plates and thin enamel but appeared to be lower crowned. They also resembled the finer-structured teeth ascribed to *Mammuthus jeffersoni,* regarded by Maglio (1973) as a northern variant of *M. columbi* in which diet has led to a convergent resemblance to the woolly mammoth. *M. columbi* is the characteristic elephant of the Sangamon and the Wisconsinan in southern North America and the lamellae are typically thicker than those of *M. primigenius* i.e. the lamellar frequency is lower; the enamel is also generally thicker.

Although morphological characters are clearly of prime importance, crown height and plate thickness are valuable statistics. Mean plate thickness can be measured as the length/lamellae ratio (crown length/number of plates) or is more usually indicated by its reciprocal, the lamellar frequency (plates per dm). A plot of the hypsodonty index against the lamellar frequency is a useful aid in diagnosis. Fig 4 shows the relative positions of the ten teeth described here in relation to the observed ranges for the upper and lower second and third molars of *M. primigenius* and *M. columbi.* All these specimens have thinner lamellae than in typical *M. columbi* and six of them lie within the observed range for *M. primigenius.* However, three good specimens (#2,4,5) have hypsodonty indexes a little below the range recorded for *M. primigenius,* adding to the impressions of Whitmore *et al.* (1967) that some of the shelf elephants may be a localized variant. The Jeddry tooth (#9) has fewer plates per 100 mm than the other specimens and is within the range for *M. columbi*; however, if the height of the unworn teeth was about 95 mm, which is probable, the hypsodonty index would lie near the *M. primigenius* field.

Another characteristic that has been employed to differentiate between these rather similar species is the thickness of the enamel and the extent of folding on the contact surface between the enamel and the cementum (Churcher 1986; Nielsen, Churcher and Lammers, 1988). In the specimens from Georges Bank the enamel is generally thin (1.4 - 2.0 mm) and moderately to finely plicate. When coupled with the lamellar frequency, all the specimens lie within the range for *M. primigenius* but some of them fall in an area of overlap between *M. primigenius* and *M. columbi.* However, at least two (#5,7) fall outside the range for *M. columbi.* As the ages of the deposits from which the teeth were derived are not certainly known, it is always possible that different ages are represented. On balance, all should probably be referred to *M. primigenius* although perhaps some individuals represent a regional or local variety. Good cranial material is needed to resolve the problem, but it is unlikely to come from the shelf. It is worth considering the teeth mentioned here as representing a partly isolated northeasternmost population of woolly mammoths occupying a tundra-like habitat south of the Wisconsinan ice sheets (Harington and Ashworth, 1986, Fig 3).

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STUDIES OF *Trichoderma* ISOLATES FROM *Mytilus edulis* COLLECTED ON THE SHORES OF CAPE BRETON AND PRINCE EDWARD ISLANDS[#][†]

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Samples of *Mytilis edulis* (mussel) were collected from coastal sites, randomly selected on the south-eastern shores of Prince Edward Island and from similar locations on the southern shore of Cape Breton Island. Cultivation of aliquots of tissue from these animals revealed a diverse fungal flora from which 49 cultures of the genus *Trichoderma* were isolated. The number of propagules from which these cultures were derived was multiplied by a factor to reflect the dry weight of mussel tissue used in the isolation. A random sample of 29 isolates from this theoretical collection was then selected and it was found possible to cultivate 28 of these in the laboratory. All nine isolates of *Trichoderma hamatum*, produced isocyanide metabolites. Improvements in the assay of these compounds are reported.

Des échantillons de *Mytilis edulis* (la moule) ont été collectionnés à locations côtières choisies au hasard sur les côtes sud-est de l'Ile Prince-Edouard et sur des lieux semblables sur la côte sud du Cap Breton. La cultivation d'aliquotes de ces animaux a révélé une flore variée de moisissures desquelles 49 cultures de ce genre ont été isolées. Le nombre de propagules qui ont fait partie de ces cultures a été multiplié par un facteur afin de réfléchir le poids sec du tissu de moule utilisé dans cette isolation.. Un échantillon de 29 isolates de cette collection théorique a été choisi, et on a réussi à en cultiver 28 dans le laboratoire. Tous les neuf isolates de *Trichoderma hamatum* ont produit des métabolirtes de l'isocyanure. On discute des améliorations du titrage de ces composés.

Introduction

In 1987 there occurred 108 incidents of poisoning (including 3 deaths) following human consumption of cultivated *Mytilis edulis* from Prince Edward Island (Bates et al., 1989). As part of the investigation surrounding this toxicity, the fungal floras of mussels from the affected areas were examined. Although no correlation between toxicity and the fungal flora was found, the diverse nature of the flora and, in particular, the predominance of *Trichoderma* spp. (Taylor, 1986) suggested an ecological examination of the populations of this genus present in mussel tissues should be done. Accordingly, the south-eastern coastal region of Prince Edward Island was divided into adjacent 1 Km^2 areas, based on the National Grid and a random sample of such areas selected. For comparison an equal area around Isle Madame, Cape Breton Island, Nova Scotia was divided and sites randomly selected in the same way. All of the areas selected that had a coastal segment were examined in October 1989 and mussels were collected from most of these areas. The fungal floras of the bulked samples were isolated and the *Trichoderma* component examined for its ability to produce antibiotics in the way we have previously described (Brewer and Taylor, 1980). The results of this work are reported.

Materials and Methods

Selection of collection areas The maps (Fig 1 and Fig 2) show the collection areas, the 9 Km^2 grids selected by the random number algorithm, and the areas where

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[†] NRCC No. 34850

[*] Author to whom correspondance should be addressed

[#] This paper is dedicated to the memory of K.A. Harrison

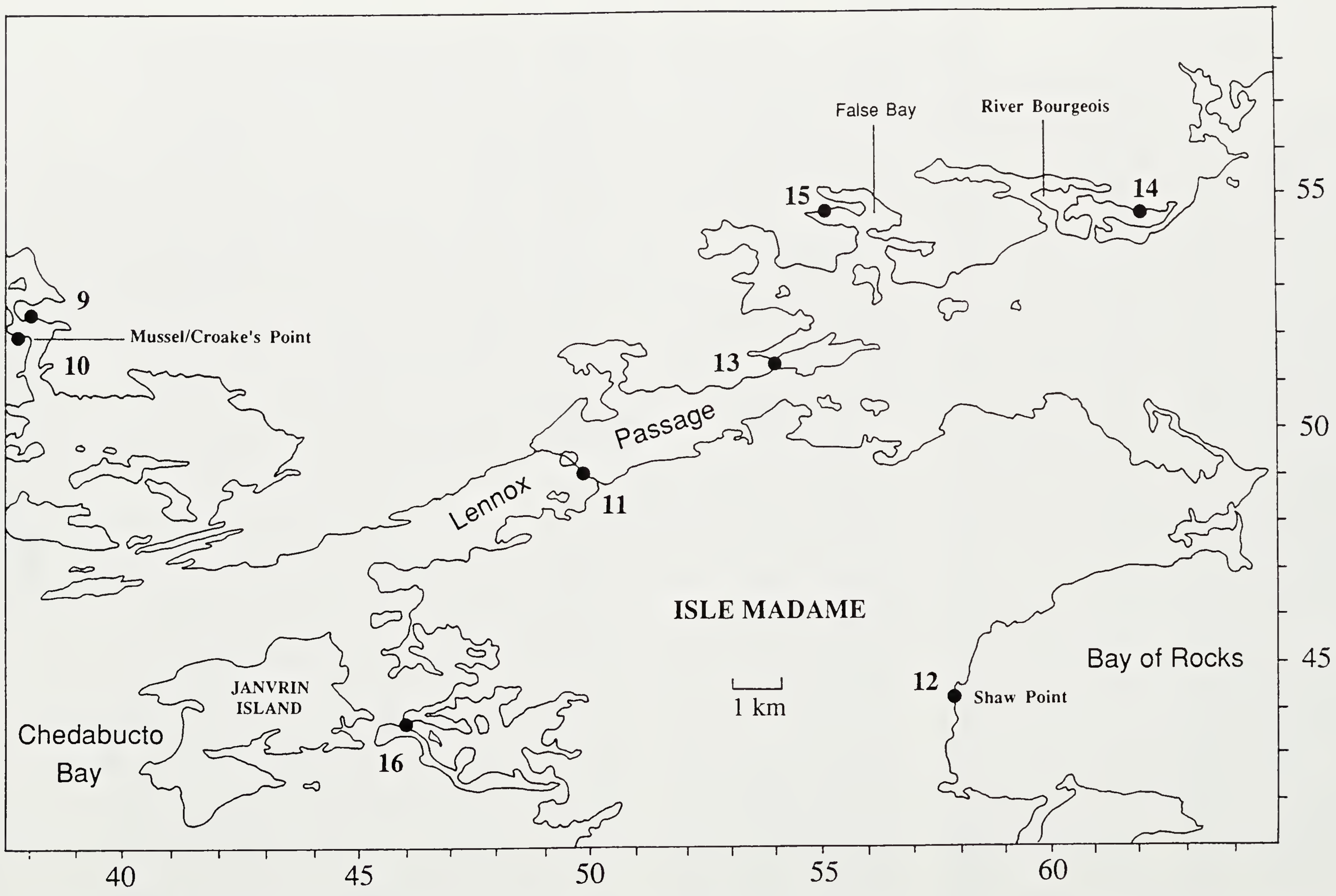

Fig 1 Map of the Southern shore of Cape Breton Island and Isle Madame. Numbers on the axes are 1 km Eastings and Northings of the National Grid. Numbers on the map refer to collection sites (Table I).

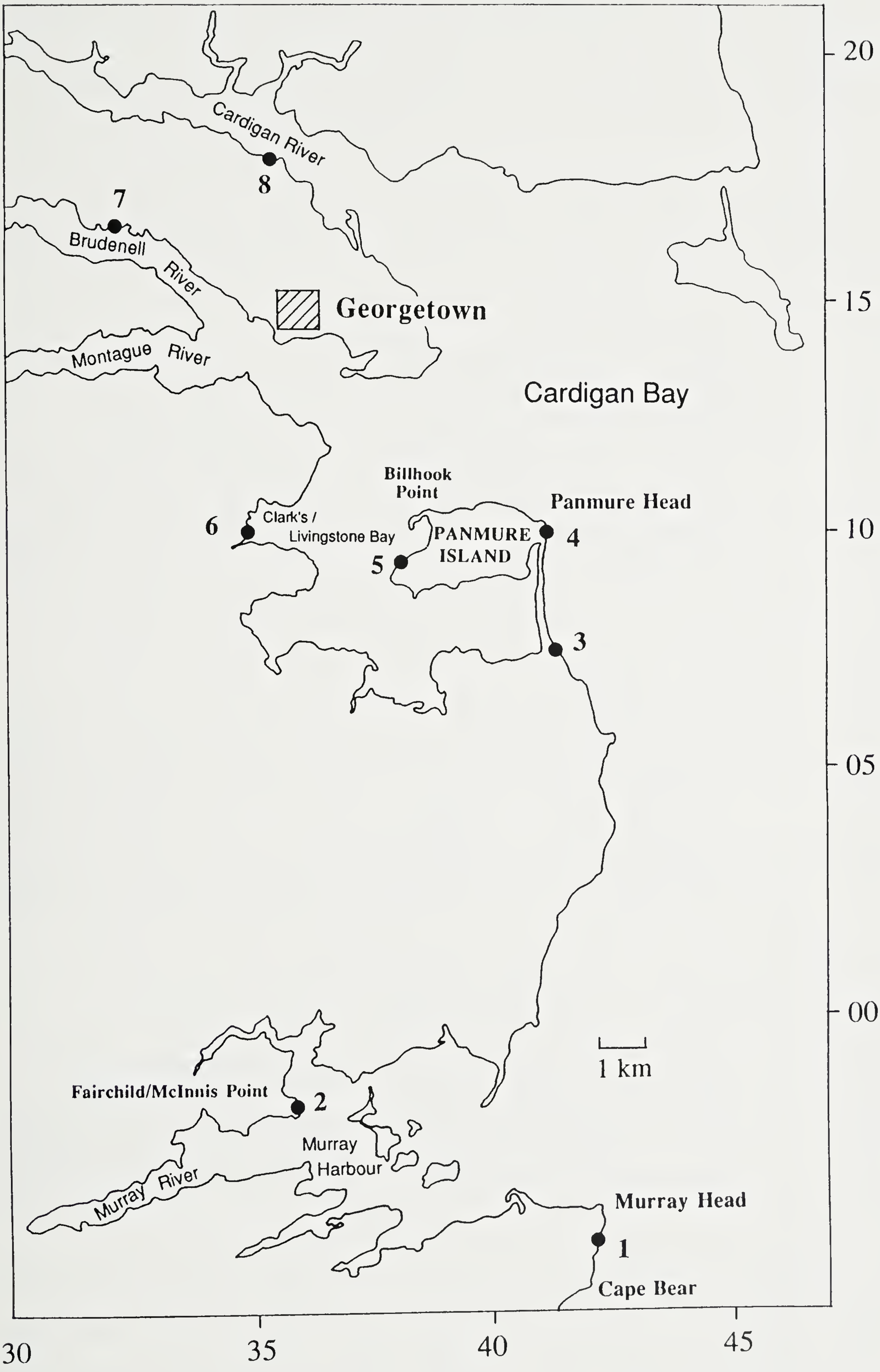

Fig 2 Map of the South-Eastern shore of Prince Edward Island. The axes and collection sites are numbered in the same way as described in Fig 1 and Table 1.

Table I Geographical locations and descriptions of areas in Prince Edward Island and Cape Breton Island where *Mytilus edulis* was collected

Reference Grid	Reference Fig 1/2	Geographical location	Nature of area of collection	Surrounding area	Day of collection (Oct. 1989)	No. of mussels examined
Prince Edward Island						
NF422953	1	Between Cape Bear and Murray Head	Rocky shore covered with *Fucus*	Road just above beach then trees and shrubs	24	13
NF359982	2	Fairchild/McInnes Point	Sandy shore - *Zostera* in water	Campground - grass and trees	24	16
NG415074	3	Smith Point	Rocky Shore - with *Fucus*	Cliffs with trees	24	20
NG412099	4	Panmure Head in front of lighthouse cliff	Rocky shore with *Fucus*	Cliff with cliff swallow tunnels	24	13
NG385093	5	Panmure Island between Billhook Point and South West Corner	Sand and pebbles	Road between scrub bush and shore - by a jetty	24	11
NG349098	6	Clarks/Livingstone Bay	Salt marsh (mud & sand)	Bush around salt marsh	24	20
NG323161	7	Brudenell Provincial Park	Sandy beach	Grass and trees	25	20
NG355175	8	East of Morrison Beach across from Ferry Point	Sandy beach	Scrub bush with cottages	25	20
Cape Breton						
PF380525	9	Exit 44 Highway 104 E of Chapel Road	Polluted beach	Road	23	20
PF382521	10	North side of Mussel /Croakes Point	Sandy beach	Camp-site & forest	23	18
PF497492	11	Lennox Passage off causeway between Burnt Is. & Benoit Is.	Side of causeway	Road/Forested island	23	18
PF579445	12	Beach, South of Shaw Point	Marsh & Tidal inlet	Road and bridge	23	21
PF539515	13	Tickle Passage	?	?	23	21
PF619546	14	"Lagoon" at River Bourgeois	Mostly mud and stones	Dirt road	24	15
PF549546	15	False Bay	Estuary; mud and stones	Mixed forest	24	27
PF463437	16	Janvin Island	Polluted and muddy	Bridge and road	24	20

M. edulis were found. In the following these areas are referred to by their National Grid numbers. These grid numbers were also used as the basis for identification of samples and of fungal isolates grown from propagules in the samples. Table I gives a list of the grid references of the areas where mussels were collected, cross references to Fig 1 and Fig 2, the number of animals used for analysis from each area, the date of collection and a general geographic description of the sites.

Isolation of fungi The mussels (Table I) were placed in polypropylene containers and were submerged in water from the collection site. Within 48 h they were transported to the laboratory where they were cooled to 4°C, opened and the tissue and fluid within the shell mascerated at 4°C for 30 s. The mascerate (10 mL) was diluted with sterile distilled water (90 mL) and 3, 10-fold dilutions prepared from this primary dilution. Each of the 3 suspensions (1 mL) was used to inoculate 10 Petri plates containing potato-dextrose agar (Difco), supplemented with yeast extract (Difco, 0.3% w/v) and rose bengal (0.0035% w/v). The plates were incubated at 25°C for 7 days, when sub-cultures of the fungal colonies on the plates were made onto a medium containing (g L^{-1}): molasses (20), dextrin (30), fish meal (15), Pharmamedia (Traders Protein Division, Fort Worth, Texas, 15) and agar (20).

Random selection of Trichoderma *isolates for antibiotic analysis* The frequency of particular *Trichoderma* isolates in mussel tissue was calculated from the number of colonies appearing at a dilution where all could be counted on 10 plates. The frequency was then defined as ((number of colonies on 10 plates x dilution)/dry weight of 10 mL of homogenate). Thus each of the 49 *Trichoderma* isolates was multiplied by this frequency, and from the theoretical collection obtained 50 isolates were selected at random. As described in a preceding paper (Brewer and Taylor, 1980), several selections were made and each selection was scored on the basis of greatest diversity of collection sites, and species, and more or less even distribution between Cape Breton and Prince Edward Islands. The selection scoring highest by these criteria had 29 different isolates from 11 collection areas, 6 from Cape Breton and 5 from Prince Edward Island.

Fermentation, Harvesting and Extraction of Trichoderma *cultures* The isolates were grown in groups of 5, together with a control culture of *Trichoderma hamatum* (HLX 1379; Brewer et al., 1982). Inocula (48 h old, 1 mL) were prepared from the isolates grown on malt agar as described in an earlier paper (Brewer et al., 1982) and were added to production medium (100 mL) containing: glucose (4 g); potassium nitrate (0.1 g);, L-tyrosine (0.1 g); sodium chloride (0.25 mg); magnesium sulfate heptahydrate (35 mg); ferrous sulfate (16 mg); zinc sulfate heptahydrate (2 mg); copper sulfate pentahydrate (2 mg); phosphomolybdic acid (3 mg); o-phosphoric acid (5.4 g) and water (conductivity <10 Ω) The solution was treated with potassium hydroxide solution to pH 5.4. Twenty such flasks for each isolate and the control were shaken in a horizontal plane at 220 r min^{-1}, each culture describing a circle of radius 3.81 cm. The culture flasks (500 mL capacity) in both fermentation stages were randomly distributed on the shaker. After 70 h at 25°C, like cultures were combined, cooled to 4°C, their pH adjusted to 8.0 when the cultures were filtered through a pad of washed 'Celite 535' from which most of the fine material had been removed by decantation. The filtrates (500 mL) were treated at 4°C with concentrated hydrochloric acid to pH 4.5 and were then extracted with ethyl acetate (3 x 150 mL). In the majority of cases the aqueous phase separated cleanly leaving an emulsoid organic phase. The 3 emulsions were combined and, if necessary, centrifuged at 1500 r min^{-1} for 30 min at 0°C. In a few cases the phases did not separate under gravity and these extractions were completed by centrifugation as described. In all cases, the combined centrifuged ethyl acetate extracts were kept at

-15°C for 18 h, when the ice that had separated was filtered and the clear filtrate (200 mL) used for analysis

Analytical methods

The dry weight of the mascerated mussel tissue was determined by drying 10 mL of the mascerate at 105°C for 24 h. Salinities of water samples were measured using an American optical hand-held refractometer; the instrument being calibrated using standard sea-water samples (Culkin and Smed, 1979). Phosphate was determined by the method of Parsons et al., (1984). All mean values are given with their standard deviations.

Antibiotic assays of fungal isolates were obtained using plugs from fungal colonies as described by Brewer et al., 1972, using *Micrococcus luteus* (HLX 701) as the assay organism.

Analysis of isocyanides in Trichoderma *fermentations* The dry ethyl acetate extract of the fermentation broth (see above, 200 mL) was treated with an ethereal solution (10 mL) of diazomethane (10 mg), the solution was kept 30 min at room temperature when the excess diazomethane was blown off with a stream of nitrogen in an efficient fume-hood. The solution was then concentrated to 20 mL and the concentrate treated with a solution (2 mL) of bis(η^5-pentamethylcyclopentadienyl)di-μ-thiocyanato-dithiocyanatodirhodium(III) (Hanson et al., 1985; hereinafter (η^5-$C_5Me_5Rh(SCN)_2)_2$; 15 mg) in methylene dichloride. The orange solution was kept 3 h at room temperature, was evaporated to dryness and the residue dissolved in methyl alcohol to give a solution of volume 2 mL. This solution (diluted as necessary, 1 mL) was injected onto a reversed phase chromatography column (Merck, C_{18}, 20 x 0.2 cm). The column was developed at 22°C with methyl alcohol at a flow rate of 1 mL min^{-1}. The column effluent was directed to a Waters UV detector set at 254 nm and the signal from the detector was integrated and plotted by a Hewlett-Packard recording integrator (Model 3854A). The remainder of the apparatus has been described previously (Russell, 1988). Standard solutions of the η^5-$C_5Me_5Rh(SCN)_2$ complexes of methyl 3-isocyanocyclopent-2-enylideneproprionate, methyl 3-(3-isocyano-1,5-epoxycyclopentyl)-prop-2-enoate, and 1-(2,3,4,5-diepoxy-1-hydroxycyclopentyl)ethanol (Boyd et al., 1991, and references therein) in methyl alcohol were chromatographed in the same way and the relations between their concentrations and peak areas obtained from the integrator were calculated. These relations were then used to calculate the yield of these three metabolites in the usual way.

The presence of isocyanide antibiotics was confirmed by thin layer chromatography on commercially prepared silica gel plates (Merck). The parent isocyanide metabolites in the esterified ethyl acetate extract were separated using the solvent acetic acid-diethyl ether-petroleum ether 0.1:29.9:70 as the developing solvent. The η^5-$C_5Me_5Rh(SCN)_2$ complexes present in the methyl alcohol solution were separated using the solvent methyl alcohol-dichloromethane 1:24 (Boyd et al., 1991).

Ultraviolet spectra were obtained using a Cary 14 spectrophotometer.

Solvents and reagents Methylene dichloride was distilled from calcium hydride before use; methyl alcohol was glass distilled and of the quality designated "for high pressure liquid chromatography". Diazomethane was prepared from *p*-toluenesulfonylmethylnitrosamide (de Boer and Backer, 1963). All other reagents were used as received.

Table II Air temperatures and physical characteristics of water at sites of collection of *Mytilus edulis* in Cape Breton and Prince Edward Island.

Grid reference	Temperature Air (°C)	Temperature Water (°C)	pH	Salinity ‰	% Dry Wt .of mussel tissue	Phosphate µM
NF422953	12	11	7.1	28	9.9	>11
NF359982	12	11	7.2	27	13	3.8
NG415074	12	11	7.1	27	13.6	5.9
NG412099	12	11	6.7	28	11.3	>11
NG385093	12	9	7.0	27	12.5	4.1
NG349098	10	12	7.3	27	9.0	0.7
NG323161	8	7	7.0	26	10.3	1.4
NG355175	8	9	7.1	26	14.2	2.3
PF380525	6.5	8.5	6.9	19	9.4	3.3
PF382521	7	8.5	6.9	19	8.1	3.3
PF497492	8	9.4	6.8	27	9.4	7
PF579445	5	10			8.0	
PF539515			6.7	27	12.9	10.7
PF619546	7.5	10	6.8	27	8.1	4.4
PF549546	6	6.5	7.6	0	8.3	3.1
PF463437	10	10.5	7.9	23	11.2	7.6

Results

A rectangle of 360 Km^2 was selected based on latitude 45° 30' 00" N and longitudes 61° 14' 46" W, and 60° 54' 3" W. The area is shown in Fig 1 and comprised most of Isle Madame, Lennox Passage and the southern shore of Cape Breton Island from Port Hawkesbury to St. Peters. This area was divided into forty, 9 Km^2 square sections based on the National grid and numbered 1-40. Sixteen of these sections were selected at random and 7 of these that had no coast-line were rejected. No mussels were found on one of the 9 sections with a coast-line.

A similar rectangle of 405 Km^2 based on latitude 46° 00' 00" and longitudes 62° 21' 00" and 62° 23' 7" was selected. The area is shown in Fig 2 and comprised the SE coast of Prince Edward Island from south of the entrance to Murray Harbour, north to Bruce Point. This area was divided into forty five 9 Km^2 square sections of which 5 (15 x 3 Km) on the SE edge were entirely oceanic. These were eliminated leaving an area the same size as that selected in Cape Breton. These areas were numbered 13-54 and 16 of these selected at random. There was no coast line on 7 of these sections, and it proved difficult to gain access to one, thus leaving 8 areas where mussels were collected.

The grid references to the nearest 100 m of the 9 Km^2 sections where mussels were found are given in Table I, and is indicated approximately in Fig 1 (numbers 1-8, Table I) and Fig 2 (numbers 9-16, Table I). Included in Table I are names of the nearest geographical location on the 1:50000 map, brief descriptions of the collection area and its surroundings, the date of collection of each sample and the number of mussels used for examination from each site. In many cases this was less than the number collected, because only shells that contained apparently healthy animals were used.

In Table II the temperatures, acidities and salinities of the water where the mussels were collected are recorded. In Cape Breton, by contrast to the Prince Edward Island sites, the water temperatures were higher than the air temperature. Temperature data were not collected for the specimens from PF539515 because this site was on private land and we were not allowed access. However the owner provided a sample which we assume was collected at this location. The water sample from site PF579445 was lost.

There appeared to be no correlation between the dry weight of mussel tissue and the pH, or the salinity, or the phosphate content of the water where they were growing. The mean dry weight of the 8 samples of mussel tissue (Table II) from Prince Edward Island was 11.7±1.8 g 10 mL^{-1} and that of the 8 samples from Cape Breton 9.4±1.8 g 10 mL^{-1}, the difference, using Student's 't' test being significant at the 90% confidence level.

In Table III calculations of the total number of fungal propagules cultivated from each gram dry weight of mussel tissue are given. The mean value of all fungal propagules on this basis cultivated from the Prince Edward Island samples was 1139±881 g^{-1}, and from the Cape Breton samples 4546±4787 g^{-1}. The very large variance in these statistics probably arises from the presence of sporing hyphae in some samples. The difference is, however, significant at the 90% confidence level. Values are also given in Table III of the numbers of propagules of 5 common *Trichoderma* spp. and in 3 cases of isolates that have been classified only to the genus level. The mean value of the frequencies of all *Trichoderma* spp. collected on Prince Edward Island was 103±113 g^{-1} and on Cape Breton 1207±1820 g^{-1}. This difference may be significant ($P<0.1$). The values obtained for *Trichoderma harzianum* (Table III) were 44 g^{-1} and 814 g^{-1} for this species in the two areas; these figures include abnormally high numbers for locations NF 422953 and PF380525. If these two values are omitted from the calculations, the mean value for the remaining 7 samples become 14±12 g^{-1} for Prince Edward Island and 224±247 g^{-1} for Cape Breton and these two means are significantly different ($P < 0.05$).

Table III Fungal propagules gram^{-1} dry weight of tissue of *Mytilus edulis* collected on the shores of Cape Breton and Prince Edward islands.

Grid reference	All fungi	*T. hamatum*	*T. koningii*	*T. harzianum*	*T. viride*	*Trichoderma polysporum*	*Unidentified Trichoderma*
NF422953	819		21	256	21		
NF359982	2282	20	32	2	2		
NF415074	456			18			2
NG412099	540	32		32			16
NG385093	723	34	17	5			
NG349098	2789	239		26			
NG323161	806	4	2	12			
NG355175	697		28	1			
PF380525	9859	235	305	4947			
PF382521	2642	247	183	148			
PF497492	1638	23		181	21		213
PF579445	1275		50	28			
PF539515	519	23	1	23	110		
PF619546	376		2	45		20	
PF549546	7229		72	505	553	48	
PF463437	12831	594	358	636	80		

A blank indicates that none of this species were found in the samples; dry weights of mussel tissues are given in Table II.

Antibiosis of Trichoderma *spp. isolated from mussel tissues* The values for the *Trichoderma* spp. given in Table III allow a theoretical collection to be assembled based on the total weight of mussel tissue collected and the frequency (number of propagules g^{-1}) of each species in each sample. This collection then represents all 49 (Table III) of the *Trichoderma* species collected and enables a random sample of this collection to be selected. The advantages and pitfalls of this procedure have been discussed (Brewer and Taylor, 1980). In this case several samples of 50 isolates were made and each was scored as described in the Experimental section. The sample chosen for study consisted of 29 different isolates, seven from five Prince Edward Island sectors and twenty-one from six Cape Breton sectors (Table IV). One of the isolates (T. *harzianum*) in the chosen sample was contaminated with a *Penicillium* sp. and was discarded. Three of the isolates of *T harzianum* in the sample could be readily distinguished from the other isolates of *T. harzianum* by the strong yellow-green pigmentation of the medium when grown on malt agar.

Table IV Frequency and antibacterial activity of a random sample of isolates of *Trichoderma* from tissues of *Mytilus edulis*

Isolate number[#]	Species	Frequency prop. g^{-1}	Activity zone diameter (mm)[*]	Trichoviridin mg L^{-1}	Dermadin mg L^{-1}	270 mg L^{-1}
NF422953-4	*harzianum*	21				
NG415074-4	*harzianum*	15	12			
NG412099-2	*harzianum*	32	8			
NG412099-20	*sp.*	16	8			
NG349098-1	*hamatum*	222	17	22	3	8
NG349098-11	*hamatum*	9	20	35	4	10
NG323161-2	hamatum	4	22	90	3	16
PF380525-1	*harzianum*	4705				
PF380525-2	*harzianum*	235				
PF380525-3	*koningii*	235				
PF380525-24	*hamatum*	235	9	10	17	66
PF382521-2	*koningii*	173			2	9
PF382521-3	*harzianum*	123			0.1	
PF382521-10	*harzianum*	25	8			
PF382521-22	*hamatum*	247	14	107		
PF497492-2	*hamatum*	9	N.D.[†]			
PF497492-3	*harzianum*	64	17			
PF497492-4	*harzianum*	43	13			?0.05
PF497492-8	*hamatum*	21	22	48	12	46
PF539515-23	*hamatum*	11	14	55		30
PF549546-2	*viride*	481				0.4
PF549546-6	*viride*	72				
PF463437-2	*harzianum*	198	8			
PF463437-3	*hamatum*	198	20	96	17	20
PF463437-4	*koningii*	198				
PF463437-8	*harzianum*	198				
PF463437-9	*harzianum*	198			?0.3	?0.7
PF463437-12	*harzianum*	40	11			

[*] *Micrococcus luteus* was used as the assay organism; blank spaces indicate that no inhibitory activity was found

[#] Characters to the left of the dash are grid references to place of isolation of the mussel sample.

[†] Not determined.

All of the remaining 28 cultures were then examined for their ability to produce metabolites that inhibited the growth of *Micrococcus luteus* and the diameter of the zones of inhibition around the plugs of fungal tissue (Brewer et al., 1972) are given in Table IV. None of the isolates of *T. viride* or *T. koningii* produced antibiotics under these conditions and only seven of thirteen *T. harzianum* isolates inhibited the growth of *M. luteus.* By contrast all 9 isolates of *T. hamatum* produced antibiotics and the mean zone diameters (16±5 mm) was greater than those observed for the active isolates of *T. harzianum* (11±3 mm, Table IV). Six of the seven Prince Edward Island isolates were active; eleven of twenty-one Cape Breton isolates were inhibitory.

Production of isocyanide antibiotics by Trichoderma *isolates from* M edulis The fermentation of isolates of *Trichoderma* from *M. edulis* and the isolation of their metabolites followed the procedures described by Brewer et al. (1982). The random sample of isolates given in Table IV were grown in five groups of 5 and one group of 3. In all six cases a control culture of *T. hamatum* HLX 1379 was included.

Several improvements in the analysis (Feicht and Taylor, 1982) of isocyanide metabolites were used taking advantage of recent improvements of our knowledge of the chemistry of these compounds. It was found that the $\eta^5C_5Me_5Rh(SCN)_2$ complexes of the metabolites (Hanson et al., 1985) had high extinction coefficients at 251 nm (1-(2,3,4,5-diepoxy-1-hydroxy-3-isocyanocyclopentyl)ethanol: ε_{251} 18500 dm^3 mol^{-1} cm^{-1}; methyl 3-(3-isocyano-1,5-diepoxycyclopentyl)-prop-2-enoate: ε_{251} 40100 dm^3 mol^{-1} cm^{-1}; and methyl 3-isocyano-cyclopent-2-enylidenepropionate: ε_{251} 12000 dm^3 mol^{-1} cm^{-1} (shoulder; λ_{max} 290 nm, ε 14200), that the methyl esters of the acidic metabolites could be prepared by the less hazardous method recently described (McAlees et al., 1990) and that these complexes (henceforth referred to by the trivial names: trichoviridin, dermadin and 270, respectively) were readily separated using a simple high pressure reversed phase chromatography system. Under the conditions described in the Experimental section, during a period of 6 months, the mean elution times observed for the 3 metabolites were: trichoviridin, 5.05±0.09 min; dermadin, 9.14±0.39 min and 270 12.20±0.4 min (n=20). All three complexes were detected and the area under the eluted peaks integrated when 20 ng of the compound was injected onto the column and in all three cases a linear relationship between weight applied to the column and integrated area was obtained (r^2 values: trichoviridin, 0.96; dermadin, 0.98; 270, 0.99). The analyses for trichoviridin were complicated when 1-(2,3-epoxy-1-hydroxy-3-isocyanocyclopent-4-enyl)ethanol (Boyd et al., 1991) and congeners were produced in the fermentation in quantities equal to or greater than trichoviridin. The former complex was eluted from the column at 4.78 min and was not completely separated from the latter. No attempt was made to find chromatographic conditions that achieved a complete separation, but the integrator was programmed to calculate the theoretical area of the trichoviridin peak. Thus the analyses given in Table IV for trichoviridin are less accurate than those for dermadin and 270 where complete separations and Gausian shaped peaks were always obtained.

The mean values obtained for the control *T. hamatum* (HLX 1379) cultures in the 6 experiments were: trichoviridin, 27±27 (range 0-80) mg L^{-1}; dermadin, 17±18 (range 0.7-56) mg L^{-1} and 270, 30±26 (range 4-92) mg L^{-1}. These values are, of course for the weights of the complexes and must be multiplied by 0.34, 0.32 and 0.306 respectively to obtain the yields of the metabolites; this also applies to the values given in Table IV. The latter values are, in general, the mean of two analyses on the same extract, but in certain cases (marked with an asterisk in Table IV) a duplicate fermentation was done. In all cases, thin layer chromatography of the concentrated ethyl acetate extract (Feicht and Taylor 1982) revealed the presence of isocyanide by their polymerization with nickelous chloride. Similarly the prepared $\eta^5C_5Me_5Rh(SCN)_2$ methyl alcohol solutions were examined by thin layer chromatography (Boyd et al., 1991); the presence of yellow/orange spots of the correct R_F confirming the presence of the three isocyanide (and many other) metabolites.

Discussion

With a base of so few examples it is untenable to draw firm ecological conclusions on relationships between *Mytilus edulis* and its fungal burden. However all samples had a fungal flora which had a striking resemblance to the soil flora from permanent pasture (Brewer and Taylor, 1980). There also seems to be some ground for concluding that the fungal populations borne by the animals from Cape Breton were greater than those from Prince Edward Island and that this may be correlated with their lower dry weight and generally less healthy appearance. Reinforcing this supposition is the fact that the samples bearing the greatest fungal populations (PF380525 and PF549546) were from the (visually) most polluted sites.

The presence of these fungal populations implies, but does not prove, a toxic potential if these animals are used as a source of food. We have made a preliminary assessment of this potential by taking a random sample of the *Trichoderma* population which is known to produce toxic metabolites *in situ* (Taylor 1986) and we found that 60% of these isolates and 100% of the isolates of *T. hamatum* produced growth inhibiting substances. These proportions are much greater than those found in isolations from soil (Brewer and Taylor 1981).

One common group of metabolites produced by this genus is characterized by an isocyanide functionality and the results of analyses of the random sample of isolates for metabolites of this type are given in Table IV. The values given and the proportions of the three compounds are only a rough guide to the production potential of these fungi - due to their great variability. This variability is exemplified by the results reported on the control culture, *T. hamatum*, HLX 1379. Whilst these results compare well with those reported about 10 y ago (Brewer et al., 1982), despite many sub-cultivations and a few selections of single spore cultures for improved production ability, it is clear that many fermentations are required to establish the mean yield of these metabolites by these isolates. Despite this proviso, it appears that only isolates of *T. hamatum* produce isocyanide metabolites. Three of the isolates of *T. harzianum* may have produced traces of these metabolites (Table IV) but subsequent repeat experiments failed to confirm this supposition. It is therefore possible that the antibacterial activity of these *T. harzianum* isolates (Table IV) is due to another group(s) of mycotoxins. This result is at variance with that reported by Fujiwara and his co-workers (1982) who found that most of the *Trichoderma* spp. given in Table III produced isocyanides.

The results suggest that when locating a site for the cultivation of *M. edulis* for human consumption the proximity of the site to roads, human habitation and sources of eroded agricultural land should be taken into consideration.

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THE OCCURRENCE OF *Pycnidiophora dispersa* IN SOIL OF A PASTURE IN NOVA SCOTIA

During the course of a study of the fungal flora of soil from a permanent pasture at the Agriculture Canada Experimental Farm at Nappan, Nova Scotia, during the grazing seasons 1973 to 1975, a number of isolates of *Pycnidiophora dispersa* Clum (= *Westerdykella dispersa* (Clum) Cejp & Milko; *Preussia dispersa* (Clum) Cain) were obtained. The location of the plots is given in Fig 1, the total area of the plots 1 to 7 being 1.9 hectares. The methods of collection of the soil samples and isolation of the fungi have been described (Brewer and Taylor, 1980).

A total of 1078 soil samples were taken from these 7 plots during the grazing seasons 1973, 1974 and 1975. The organism, *Pycnidiophora dispersa,* was isolated from 53 of these soil samples. That this organism was isolated from soil is not surprising, as it has been isolated from soils in Cambodia, Nigeria, South Africa, U.S.A., Pakistan (Cain, 1961; von Arx & Storn, 1967), Hawaii (Lee and Baker, 1972) and Honduras (Goos, 1963). Additionally isolates from soils in India, Armenia and Holland are given in the catalogue of the International Mycological Institute (10th Edition, 1992). Thus this species is a common, world-wide, constituent of soils.

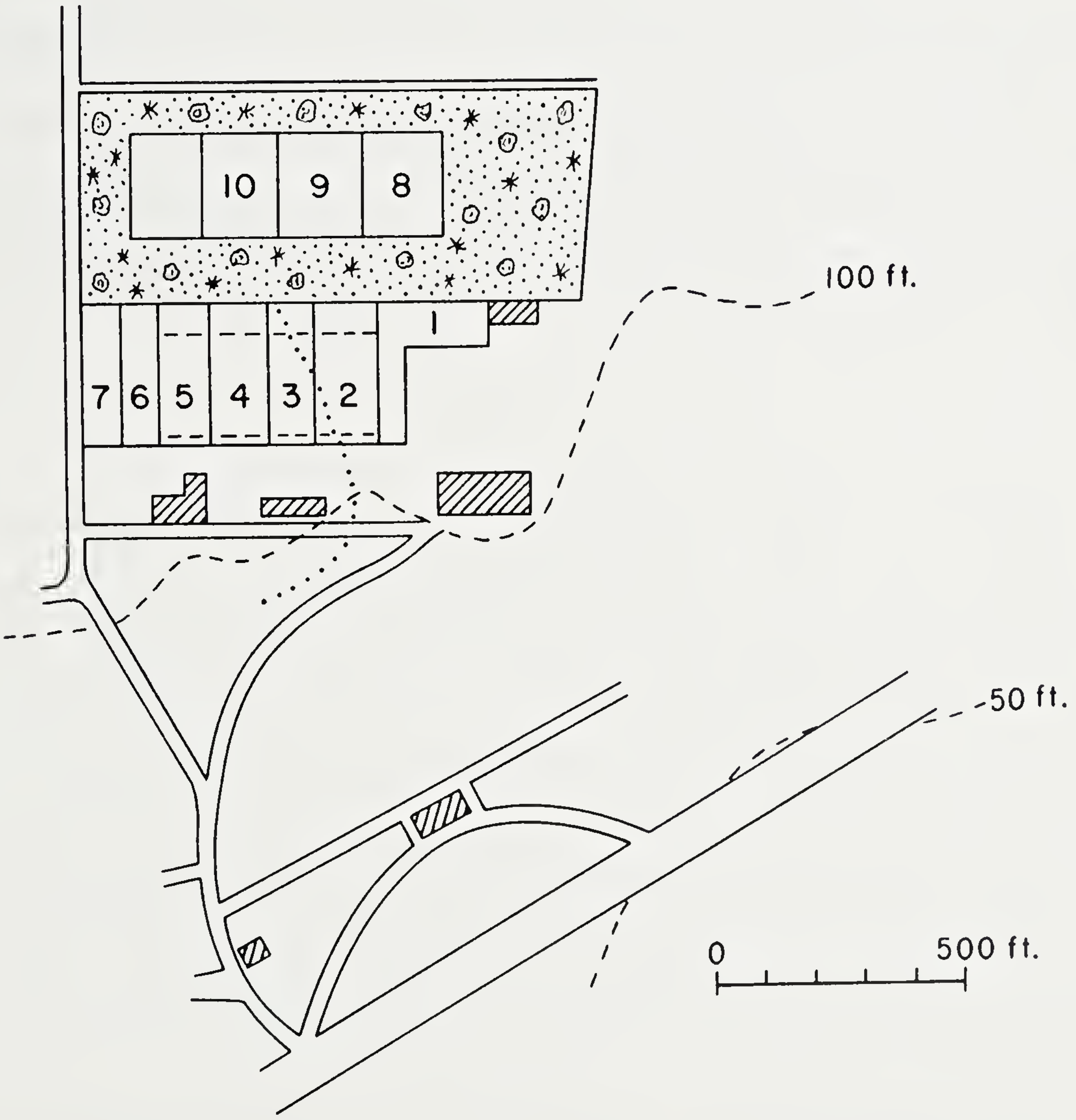

Fig 1. Location of experimental plots at Nappan, Cumberland County, Nova Scotia; the road at the 50' contour is route 302.

However, an interesting point that emerged from the collections at Nappan was that this organism was only isolated from soil samples from plots 6 and 7, comprising approximately 4800 m^2, from which 267 samples had been collected. Fifty two of these samples (19.5%) yielded this fungus. The number of samples collected from each sector and the number from which the fungus was isolated are given in Fig 2. The 10 sectors of each plot were of equal size. There appears to be a concentration of isolations of *P. dispersa* in sectors 6,8 and 10 of plot 6 and sectors 5,7,8,9 and 10 of plot 7. The area of the sectors 6, 8 and 10 of plot 6 and 5, 7 and 9 of plot 7 corresponds approximately to the location where grass silage had been made for a number of years prior to ploughing and re-seeding in 1972. The area covered by sectors 8 and 10 of plot 7 includes the route taken by the vehicles transporting the grass to the pile and removal of the silage.

Over the years from 1967 to 1971, 567 samples were collected from an area delimited by plots 2, 3, 4, and 5 to the broken lines in Fig 1. There were no isolates identified as *P. dispersa* amongst the fungi isolated. Soil samples were collected in the years: 1971 (21 samples), 1974 (150) and 1975 (156) from the area depicted as plots 8, 9 and 10 in Fig 1. and again no isolates identifiable as *P. dispersa* were obtained from these samples. Similarly, no isolate of this fungus was obtained from 430 soil samples obtained over the years 1967-69 and 1971 from a permanent pasture somewhat removed from Plots 6 and 7 (Plot R3, Brewer *et al.* 1971), nor from samples collected in 1971 from a corn field (28 samples) and a hayfield (28) on the Experimental Farm.

It appears that the fungus *Pycnidiophora dispersa* is limited to the location of plots 6 and 7 and particularly concentrated in the area where grass silage was made previous to ploughing and re-seeding. It is possible that some of the isolates collected elsewhere, that failed to either sporulate or produce a teleomorph in culture were non-fruiting non-sporulating isolates of *P. dispersa,* but that would in no way detract from the interesting observation of the apparent influence of the grass silage pile on the possibility of obtaining identifiable isolates of the fungus from the underlying soil.

PLOT 7	2 0/5	4 0/11	6 2/12	8 2/8	10 3/7
	1 0/12	3 0/14	5 2/5	7 7/14	9 6/13
PLOT 6	2 1/10	4 3/19	6 5/21	8 11/17	10 5/11
	1 0/17	3 0/17	5 0/12	7 3/22	9 2/20

Fig 2. Sectors of plots 6 and 7. The numbers in the upper left hand corner of the segments in the diagram are used for reference in the text. In the centre (x/y) are given the number of soil samples from which *Pycnidiophora dispersa* was isolated (x) and the total number of samples collected from the sector (y).

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Adiantum pedatum IN NOVA SCOTIA

Adiantum pedatum Linneus, endemic to North America is the northernmost element of the genus *Adiantum*, a large diverse group of tropical ferns. Plants of this complex are distinguished from other north temperate members of the genus by their pedate leaf architecture (Paris and Windham, 1988). There are four North American subspecies of *A. pedatum*: *calderi* Cody; *subpumilum* W.H. Wagner; *aleuticum*; and *pedatum* (Paris and Windham, 1988; Cody and Britton, 1989). The woodland maidenhair fern, subspecies *pedatum*, is a diploid taxon morphologically and isoenzymically distinct from the diploid eastern serpentine subspecies *calderi* and other western subspecies (Paris and Windham, 1988).

The earliest record of *Adiantum pedatum* in Nova Scotia is that of Ball (1876), who reported that it had been found in Newport, Hants county and could be seen growing in gardens as an ornamental plant. Lawson (1889) reported an additional wild location at Upper Musquodoboit, and Robinson (1904) another from Cape Breton. Roland (1941) gives five locations in addition to those at Newport and Upper Musquodoboit - Yarmouth county, Canaan, Kings county, Clarkesville and Scotch Village, Hants county and East Mountain, Colchester county. von Aderkas (1987) gives the locations of four specimens in the collection held at Acadia University and there are two specimens in the Nova Scotia museum (not reported by von Aderkas, 1987).

All of these authors emphasise the beauty and rarity of *A. pedatum*. It is described as rare in the Flora of Nova Scotia (Roland and Smith 1969) and it is found in the catalogue of rare vascular plants of Nova Scotia (Maher et al., 1978).

Adiantum pedatum was found on June 14, 1989 during a study of the plant community of the Meander river intervale, near Brooklyn, Nova Scotia (45° 01' 13" N, 64° 03' 40" W). This site is a small remnant of old hardwood forest dominated by species of the Alleghenian floral element maintained on an alkaline soil (von Aderkas and Bird, 1983). The plant was found in one location growing vigorously from forked horizontal rhizomes and possibly represents a single genetic individual. Close associated plants at the site included *Onoclea sensibilis* L., *Matteuccia strutheropsis* (L.) Todaro, *Sanguinaria canadensis* L. and *Acer saccharum* Marsh. The forest is generally open in the vicinity, the nearest tree was an ironwood (*Ostrya virginiana* (Mill.) K. Koch and the canopy comprised of *Quercus borealis* Michx., *Populus balsamifera* L. and *Acer saccharum*.

Voucher specimens have been deposited in the Herbaria of Acadia University, Wolfville (ACAD ECS 014277) and the Nova Scotia museum (No accession number)

Acknowledgments

I thank Drs. T. Herman and S. Van der Kloet and A. Wilson and R. Newell for their help.

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Obituary of K.A. Harrison

K.A. Harrison was born on a farm in the St. John river valley about 16 km east of Fredericton in 1901 and died at Kentville Nova Scotia on November 5 1991. Like many other notable biologists from the Maritime Provinces, his education in applied biology started at the Nova Scotia Agricultural College and continued, specializing in horticulture, at the Ontario Agricultural College where he obtained a B.Sc in 1924. He then read plant pathology at Macdonald College, studying anthracnose of beans. Harrison noted that beans, a minor crop, were worth in 1917-1921 about $502,000 in Nova Scotia (In 1980 (anonymous, 1980) their value was $78,000!).

These studies, published in his early twenty's, show a remarkable grasp of the complexities of fungal infestation of plants and notably include investigations of material from his home in Maugerville, New Brunswick. The data presented in these papers clearly show the range of virulence of different isolates of *Colletotrichum lindemuthianum* on some 65 cultivars of *Phaseolus* spp.. The papers are also remarkable because of the methods described for study of the growth of these isolates in the laboratory - methods used with little modification or acknowledgement, twenty years later during the development of penicillin manufacture.

Harrison joined J.F.D. Hockey at the Kentville Research Station in 1926. Hockey had been asked to start a plant pathology unit at the Station in response to demands by the horticultural industry in the Annapolis Valley. The two botanists created a laboratory that not only provided a service to growers in Nova Scotia but also initiated many new methods of forecasting outbreaks of disease and of disease control. In the case of predicting onset of disease, they were among the first in the world to use the sticky microscope slide spore trap. This instrument is mounted so that an aliquot of air in for example an orchard, impinges on the slide when the particles in the impacted air stick to the slide. The population densities of fungal spores - the agents of disease transmission, can then be determined microscopically. When the slides were exposed over leaves, in moist conditions, the trap caught spores of *Venturia inaequalis* as its ascospores exploded and thus provided a rational method of predicting the spread of apple scab caused by this fungus. An important offshoot of this spore trap work resulted from the exposure of the trap in orchards where it captured apple pollen. This demonstrated for the first time that wind was an important factor in pollination in apples, and also permitted estimations of pollen densities and hence the efficiency of pollination in orchards.

Harrison's work on diseases of horticultural crops and in stored fruit and vegetables spanned almost 40 years. Its range, so far as fungal diseases are concerned, was wide and a number of examples are worth giving to illustrate this and, hopefully to stimulate further work. Early taxonomic work on *Gloeosporium* and its teleomorph *Physalospora miyabeana* revealed the different susceptibility of different willow species to this fungus. Despite the known presence of many antibiotic metabolites in *Salix* spp. an adequate explanation of this phenomenon is not available and even the fungus is rare either as an Herbarium specimen or as a live culture (Brewer et al. 1991). Studies on storage problems revealed that these were frequently due to fungi, benign in the growing fruit, but capable of rapid growth during storage. This led to investigations of the mode of penetration and survival of such organisms, and a few mechanisms for these phenomena were proposed, though none substantiated - all aspects of such dormancy and growth stimulation remain obscure (see e.g. Wheeler, 1968).

The advent of chemically synthesized fungicides led to work on the control of fungal diseases in tomatoes, raspberries, and cranberries. This work, carried out in the 1950's and published in the period 1960-1968, reveals Harrison's understanding of the importance of the design and the (sceptical) use of statistical methods in the analysis of results of plot experiments. The results obtained were of use to commercial horticulture and Harrison's ability to write for two audiences - his scientific colleagues and working horticulturalists was an art many could profitably emulate. His pamphlet on cranberry diseases and their control has run through several editions.

The Basidiomycetes are a large group of plants devoid of photosynthetic pigments. They are collected for their medicinal properties (Kanematsu and Natori, 1970; Kubota et al., 1982)), as a source of dyestuffs (Stahlschmidt, 1877) and many are highly regarded for their nutrient and flavour constituents (Groves, 1975). However, every year, they are the cause of many cases of poisoning, mostly because of the difficulty of distinguishing toxic species e.g. the similarity of certain species of *Cantharellus* and *Clitocybe*. It is important to understand that problems of mistaken identity are not trivial - the professional mycologist is only less likely to be the victim of the taxonomic complexities of the Basidiomycetes (and other fungi).

Harrison was an enthusiastic collector of field mushrooms throughout his life and his knowledge of mycology made him a natural leader of forays, well able to provide expert taxonomic advice. On his retirement in 1966 he was able to greatly extend his studies of the taxonomy of the Hymenomycetales and in particular the Agaricaceae, Hydnaceae and Boletaceae. His many contributions to this speciality are well illustrated by a contribution to the Proceedings on Nova Scotian *Amanitas* - some poisonous, others not. In this paper the first collections of *A. wellsei* and *A flavorubus* var *gracilis* in Nova Scotia were reported. The paper contains beautiful photographs of the five species described, photography characteristic of Harrison's skill, often demonstrated in lectures he gave to the Institute, notably one in 1953 when 86 colour slides of 21 families of Basidiomycetes were shown. Some of these have been published (Groves, 1975; Ammirati et al., 1985).

However it is probably his studies on the taxonomy of the Hydnaceae that most characterise his work in the period 1966-1990. The basis for these studies was numerous field trips to, the Great Smokey Mountains on the Tennessee-N. Carolina boundary, New Mexico, the Michigan peninsula and the Pacific coast of Washington, Oregon and British Columbia. Much of this work was done whilst he was a research associate of A.H. Smith at the University of Michigan herbarium at Ann Arbor. During this time he described new species of *Steccherinum*, *Phellodon*, *Hydnum* (5 spp.) and *Hydnellum*(10 spp.), and contributed a taxonomic key and descriptions of the Hydnaceae. This work revealed many taxonomic uncertainties, particularly with respect to correspondence between European classification (Maas Geesteranus, 1971, 1975) and that proposed for N. American isolates. These uncertainties were reviewed in detail by comparison of the colour reproductions in Maas Geesteranus's monograph (1975) with Harrison's collection of Kodachrome photographs. It is clear, from the many discrepancies that much taxonomic work remains, though it seems unlikely that problems will be resolved on the basis of coloured or olfactory metabolites. Harrison was aware of these difficulties and towards the end of his life initiated investigations into the isoenzymes laccase and tyrosinase as taxonomic markers in the Basidiomycetes. Much necessary preliminary work was done to show that these markers were insensitive to the age of the fungal material, storage times or geographical location of the collection sites (Nova Scotia and New York State). Two species, *Hericium* *americanum* and *H. ramulosum* were shown to be laccase positive and thus the stage seems to be set for a more objective taxonomy of the Hydnaceae.

Kenneth A. Harrison was a patriot who served his country with its armed forces in wartime and his fellow citizens in peace-time as a teacher, a professional plant pathologist and as an example to be emulated. It is entirely appropriate that Acadia University has dedicated a mycology laboratory in his honour. His many colleagues and friends mourn his loss and extend their condolences to his family.

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PROC. N.S. INST. SCI. (1993)
Volume 40, pp. 53 - 54

Turing Topics

This column is intended to be a permanent feature of the Proceedings of the Institute. It has been started because the emergence of reliable 3.5" magnetic discs, capable of recording $>10^6$ characters is having a great effect on all aspects of publishing by scientific societies. This format is not only cheaper to produce than traditional printed journals, it is in many ways more useful to the working scientist because of its greater flexibility. Users can add, delete, modify and search for information on these discs very easily, the discs can be copied and stored in a minimum space.

The Institute proposes to publish work on this medium in three fields:

A Each issue of the Proceedings will be available on these discs from Volume 40 Part 1 (this issue) onwards. Members of the Institute will be able to decide whether they wish to receive their copies of the Proceedings in the normal printed form or on 3.5" or 5.25" floppy discs as part of their membership dues. (They will be able, of course, to purchase the other format.) Authors who wish to publish data that normally would be deposited in a databank e.g. crystallographic data, field observations etc. are encouraged to incorporate such extensive data with their typescripts. It will be published by the Institute and deposited in the Institute's library in the magnetic disc format - only the typescript part will be printed.

B In the past the Institute has published encyclopaedic lists of plants and animals characteristically found in the Maritime Provinces. In future this sort of material will also be published in the floppy disc format. At the moment the following discs are available:

1. The cumulative indices (volumes 1-39), Author & Subject, of the Proceedings (see *Proc. N.S. Inst. Sci.*, 39: 149). The disc includes a utility that searches the author index for all a given author's publications in the Proceedings, giving titles and references.
2. Indices of available fungal cultures. These indices are lists of cultures available from culture collections and include the following details of each culture: binominal name, accession number, the substrate and place of origin of the fungus, details of its maintenance and its toxicity (see *Proc. N.S. Inst. Sci.*, 39: 79). The following collections are available:
   a Agriculture Canada (DAOM Collection ≈7500 cultures)
   b. Forintek Ltd., Canadian Forestry Service, in Fredericton, and in Edmonton (Northern Forest Research Center) and Centre de Recherche en Biologie Forestière, Laval University (≈4000 cultures)
   c. Cultures available from the National Research Council (some are being transferred to Agriculture Canada; ≈2000 cultures)
   d. Yeast cultures from Labatt's Ltd and a few other sources (≈600 cultures)
   e. Cultures available from the International Mycological Institute, United Kingdom (≈9200 cultures).

All of these are sold with instructions for their use and a programme for searching the data.

3. A list of culture collections; their curators, holdings (bacteria, fungi, algae, virus etc.) and addresses

All of this material is available from the Institute's Librarian, Science Library, Dalhousie University, Halifax, Nova Scotia B3H 4J3 at a cost per disc of $25.00 for individual scientists and $CAN 50.00 for institutions. In ordering please state the data format (IBM or Macintosh, 3.5" (0.8 or 1.44 Mbytes), 5.25" (.6 or 1.2 Mbytes) required.

C Inevitably the first occurrences of this column will be dominated by news like that given in the preceding parts, but we hope that this will be a temporary phenomenon, because this column is intended for the use of members of the Institute. As more members use and design computer software its utility, accuracy and value will become apparent to them. It will benefit all of their colleagues if they publish these experiences, good and bad, in this column. Such publications should be succinct, and they will be reviewed. They will be published under their author's name and will be restricted to 500 words.

The information in this and succeeding columns will be accumulated on a floppy disc which will be available to members. We hope that a mechanism will evolve whereby members can keep their records of the column current at minimum expense to themselves.

PRESIDENT'S REPORT 1992-93

Over the past year the Institute presented the following five lectures and two panel discussions:

October:	Biomechanics Applied to Lifting Problems in Industry. John Kozey, Kin-Erg Consulting & Technical University of Nova Scotia
November:	Systems are for People. Peter Hitchcock, Technical University of Nova Scotia
December:	Form and Function of Large Whales. Paul Brodie, Bedford institute of Oceanography
January:	Geomatics: Technology Trends and Applications. Gerry Post, Advisor to the Maritime Geomatics Board
February:	A Comprehensive Science Strategy. S.B. MacPhee, G. Smith, R.Foxall, K. Ogilvie
March:	Body-surface Potential Mapping. Cheryl Kozey, School of Physiotherapy, Dalhousie University
April:	Quality of Research and Implications for Funding. A. Bowen, J. Halliwell, A. Marble, D. Pink

Attendance at the lectures drew, on average, around thirty per meeting. The panel discussions format was an innovation which, judging by the attendance and comments, was quite successful. It is intended that more panel discussions be incorporated to cover topical items such as science education, governmental and political concerns for the role of science, environmental issues and the absorption of science into everyday life. The council is making a determined effort to ensure that the Nova Scotian Institute of Science fulfills its role as a scientific forum.

In addition to the list presented above, Dr. K.H. Mann of the Bedford Institute of Oceanography gave the Opening Public Lecture of the Association of Science Teachers at their Annual Conference held in October. In the lecture entitled "Physical Oceanography, Food Chains and Fish Stocks" Dr. Mann illustrated the practice and interaction of biology, chemistry and physics as applied in the fields of ecology and oceanography and showed how these several disciplines have been interwoven to follow and explain the dynamics of life in the ocean.

Over the course of the year the Council has discussed our membership numbers and the need to ensure that the community was well represented in the Institute. To that end special efforts were made through a membership drive and by seeking the advice of the Institute through a questionnaire. The treasurer's report indicates the success of the membership drive whereby a substantial number joined or renewed their affiliation with the Institute. Dr. Davis prepared a questionnaire to obtain the opinions of the Institute members as to the kind of activities they wished to see; he has summarized the responses for presentation to this meeting. They will be used by the Council to guide their proposals for Institute programmes. In addition, the Institute has attempted to improve the contacts with the Science Teachers of Nova Scotia and wishes to have one of their members attend all council meetings as an observer.

As in previous years the Institute has maintained its publications as a mode of communication. It should be noted that the Institute Proceedings has been of paramount importance especially in providing an outlet for works on the flora and fauna of Nova Scotia. The Editor's report has reviewed the publications for the year and mentions in particular the major contribution Institute members have made, through the Proceedings, to the Natural history of Canada and the Maritime provinces. Volume 39 of the Proceedings was produced this year. The Librarian's report has described the collection which is held at the Science Library at Dalhousie University. I wish to acknowledge the support offered by the staff of Dalhousie University and our appreciation for the efforts of both the Librarian and Editor.

I wish to thank the Nova Scotia Museum who have provided financial support for the Institute Proceedings and for its provision of space and facilities for the monthly meetings of the Institute.

Finally I wish to thank members of the Council of the Institute who have been generous in giving their time and effort on behalf of the Institute. Their support has made my time as President most enjoyable.

James E. Stewart

NOVA SCOTIAN INSTITUTE OF SCIENCE
BALANCE SHEET
AS OF MARCH 31, 1993

ASSETS:

Current Account	$40.34		
Savings Account	$966.31		
Wood Gundy Account	$938.26		
Petty Cash	$57.35		
TOTAL CASH		$2,002.26	
INVESTMENTS		$33,582.65	$35,584.91
ACCOUNTS RECEIVALBE			$566.14
TOTAL ASSETS			$36,151.05

LIABILITIES AND NET WORTH:

ACCOUNTS PAYABLE			$261.61
DEFERRED REVENUE			$10.00
PERMANENT FUND		$5,346.34	
SURPLUS AS OF APRIL 1/92		$31,669.60	
EXCESS INCOME/(EXPENSES)		($1,136.50)	$35,879.44
TOTAL LIABILITIES AND NET WORTH			$36,151.05

INVESTMENTS:

Maturing May 20/93 @ 5.40%		$33,582.65	
			$33,582.65

RECEIVABLES

GST Refundable	$182.21	
Deposit Apr. 16/93	$215.00	
Interest	$168.93	
		$566.14

PAYABLES

Valley Chapter	$112.00	
New Leaf Enterprises	$126.09	
Dalhousie University	$23.52	
		$261.61

INSTRUCTIONS TO AUTHORS

The *Proceedings of the Nova Scotian Institute of Science* publishes the proceedings of the Institute and original articles, including notes, pertaining to the natural sciences of the Atlantic Provinces, especially in biology and geology. Manuscripts may be either in English or French, and acceptance for publication is based on recommendations of referees.
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Spelling should follow that of **Webster's Third New International Dictionary**, and authors are responsible for consistency in spelling.

Abbreviations, nomenclature, and symbols for units of measurements should follow international recommendations. Metric units, SI units and decimals should be used whenever possible. Use day/month/year sequence for dates. Do not use periods after such abbreviations as "mm, kg, DOE, NRC, etc."

Taxonomic keys should be in aligned-couplet form in zoology and paleontology, and in multi-level indent form in botany. Synonomy in botany, zoology, and paleontology should be in the short form—taxon, author, year, page—with full citation in the references.

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Volume 40 1995 Part 2

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EDITORIAL NOTE

In addition to regular papers authors are invited to submit short papers to the *Proceedings* as Brief Communications. Originally these were not required to have abstracts but, in order to be listed in *Biological Abstracts* and similar publications, abstracts are now required. Abstracts should be in French and English. Assistance with transaction will be provided by the Institute when required.

All manuscripts should be prepared according to the instructions for authors provided inside the front and back covers of the *Proceedings* (see Vol. 40/1). The instructions are currently under revision and have been omitted from the current edition. Manuscripts should be sent to the Editor, Dr. D.S. Davis, 10 Forest Road, Dartmouth, Nova Scotia, B3A 2M3, Canada.

ERRATUM

Volume 40, Part 1, Page 25, Figure 3. In the caption the letter pairs A, B and C, D have been transposed. The caption should read as follows: A,B: Right lower third molar, found by Victor Joddry (#9) seen in outer lateral and occlusal views. Note abnormal asymmetrical concave wear surface. C,D: Left upper third molar, YCM NS 92.6 (#10), in the Yarmouth County Museum collections, seen in outer-lateral and occlusal views. One third natural size.

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MIGRATION OF *Salvelinus fontinalis* IN RELATION TO THE DATE OF SPRING THAW (ICE-OUT) OF EIGHT ISLAND LAKE, GUYSBOROUGH COUNTY, NOVA SCOTIA*

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In the period 1965-1992 the mean difference in days between the spring thaw ("ice-out") of Eight Island Lake in Guysborough County, Nova Scotia and the first catch, by sport fishermen, of *Salvelinus fontinalis* was 11.5±2.7., despite the spread of ice-out during the same years, over a period of 43 days (March 24 - May 6).

Pendant la période de 1965 à 1992, la différence moyenne entre le dégel printannier (ice-out) du Lac Eight Island du comté Guysborough en Nouvelle-Ecosse et la première prise de *Salvelinus fontinalis* était 11.5±2.7 jours, malgré l'écart du dégel pendant les mêmes années à travers une période de 43 jours (du 24 mars au 6 mai).

Salvelinus fontinalis, hereinafter called "sea trout" to distinguish fish that had migrated to salt water, is an anadromous char which spawns in fresh water like other salmonids (Livingstone, 1951). In this species spawning usually occurs in the fall. Unlike *Salmo salar* these fish do not forage into the ocean and populations of the various river/bay systems tend to remain separate. Thus rivers have characteristic periods for the annual return of sea trout.

This paper presents data gathered since 1965 on the run of sea trout up the Country Harbour River, Guysborough County and its relation to the times of ice-out of Eight Island Lake (45° 20'N, 61° 57'W; Alexander et al., 1986).

Details of the catch of all trout made from two fishing camps have been recorded since 1965. Fishermen used a variety of lures, bait etc. and the fishing started when the lake cleared of ice or the legal fishing season began - whichever was later. There are gaps in the data, partly due to bad weather and the absence of early fishing trips

The data are collected in Table I

Table I Dates of ice-out and the first catch of sea trout (*Salvelinus fontinalis*)

Year	Date	
	Ice-out	First catch
1965	May 1	May 15
1967	May 6	May 19
1968	April 7	April 20
1969	?	April 19
1973	April 21	May 3
1975	May 3	May 11
1976	March 24	?
1977	April 27	?
1981	March 28	April 11
1983	April 6	April 16
1986	April 7	April 18
1987	April 18	?
1990	April 15	April 19
1991	April 28	May 9
1992	May 2	May 15

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* Dedicated to the memory of Walter J. Chute

† Author to whom correspondence should be addressed

In general the fish caught were about 30 cm in length (largest recorded 40 cm). They were slim, silvery sided with very pink flesh (some trout caught were not - presumably they had been in the lake over the winter and they are not included in the Table).

The ice cleared from the lake usually in a matter of hours and its timing was therefore more precise than the date of the run of the first sea trout. Nevertheless the mean value of this time interval is about 11.5 days with a standard deviation of less than 3 days, this despite the fact that the onset of the run was unlikely to be detected in all years. By contrast the date of ice-out was more variable and over the 27 years this varied from March 24 to May 6.

This early migration of *S. fontinalis*, 16 Km up the Country Harbour River may be compared to the July (anecdotal among sport fishermen) migration of the species up the Musquodoboit River, also draining into the Atlantic Ocean and June and July migration up the Dunk River in Prince Edward Island (Johnston and McKenna, 1976). It seems possible that this early migration reflects a survival strategy related to the shallow depth of the Country Harbour River and the small streams that drain into Eight Island Lake from lakes higher in the watershed. By contrast, the Musquodoboit River is deeper, and the Dunk River is shorter and close connected to salt water

The factors that stimulate this migration are unknown but it seems unlikely to be due to light intensity since the onset of the run is seasonably variable. A more plausible factor might be the water temperature since this is probably higher earlier in the season in this shallow river as compared e.g. to the Musquodoboit.

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THE BIODEGRADATION OF L-TYROSINE BY *Trichoderma hamatum* TO TRICHOVIRIDIN AND RELATED COMPOUNDS[#§]

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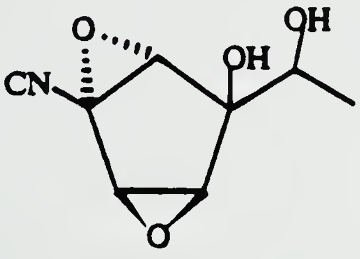
Trichoviridin (**1**) is shown to be a degradation product of L-tyrosine by cultures of *Trichoderma hamatum*. The specific radioactivity of trichoviridin thus formed was lower than that of 3-isocyanocyclopent-2-enylidene propionic acid (**2**) a congenic catabolite which contains an additional labelled carbon atom from U-^{14}C-L-tyrosine. Evidence is presented that two other neutral isocyanides produced by the fungus are also degradation products of tyrosine.

On a démontre que le trichoviridine est un produit de dégradation de L-tyrosine dans les cultures de *Trichoderma hamatum*. La radioactivité spécifique du trochoviridine obtenu ainsi est plus basse que celle de l'acide propionique de 3-isocyanocyclopent-2-enylidine, un métabolite congénère qui contient un atome de carbone étiqueté supplémentaire obtenu de la U-^{14}C-L-tyrosine. On présente l'évidence que deux autres isocyanures neutres produits par ce mycète sont aussi produits de dégradation de la tyrosine.

The biosynthesis of the acidic isocyanide (**2**) has been shown to involve the degradation of the amino acid L-tyrosine by cyclization of the side chain and oxidative fission, with loss of one carbon atom, of the phenolic ring (Baldwin, et al., 1985).

The antibiotic **2** is one of about 15 congeners (Boyd, et al., 1991), many of which are neutral C_8 entities (Fujiwara, et al., 1982). The most abundant of these bears the trivial name trichoviridin (**1**); its isolation and structure were reported by Nobuhara et al., (1976). The similarities in the structures of metabolites **1** and **2**, which both contain a five-carbon ring bearing an isocyano substituent and a side chain in a 1,3 relationship suggest that trichoviridin may be the result of further biological degradation of L-tyrosine.



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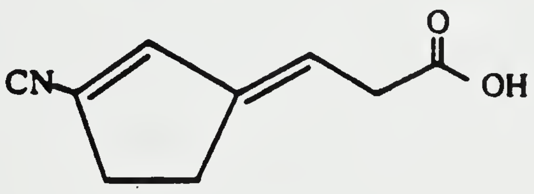

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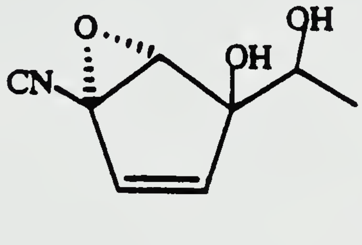

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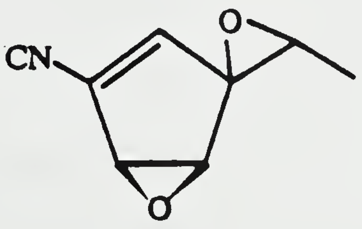

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U-^{14}C-L-Tyrosine (203 μCi) was added to a *Trichoderma hamatum* fermentation on a salts-glucose defined medium (Brewer et al., 1982) 24 h after inoculation and the culture was harvested 48 h later. The acidic isocyanide (**2**, 20 mg L^{-1}, ε_{270} 12000 dm^3 mol^{-1} cm^{-1}) was isolated in the usual way (Brewer et al., 1982) and its specific radioactivity was 2.76 mCi mmol^{-1}, representing a recovery of ^{14}C of 2.04%.

The ethyl acetate raffinate from which the acidic catabolites had been removed was radioactive (4.19 μCi = 2.06% of the radioactivity added to the fermentation). Chromatography gave three radioactive fractions which collectively accounted for 90% of the radioactivity of the ethyl acetate solution. Analytical thin layer chromatography showed that the fraction of greatest R_F co-chromatographed with trichoviridin. Colorless crystals (28.1 mg, m. p. 89-92°, 1.9 μCi mmol^{-1}) were obtained by slow evaporation of this fraction; they were collected and recrystallized from diethyl ether-hexane giving trichoviridin (**1**, 21 mg, m. p. 92° 1.82 μCi mmol^{-1}). This material eluted as a single Gaussian shaped peak on reversed phase partition chromatography of its Rh{η^5-$C_5Me_5(SCN)_2$} complex (Brewer et al., 1993), and its specific radioactivity was unchanged upon further recrystallization.

This trichoviridin was further characterized by reaction with veratric acid and dicyclohexylcarbodiimide in the presence of a trace of 4-dimethylpyridine (McAlees et al., 1990). The crystalline ester obtained had a single sharp absorption band at 3560 cm^{-1}; by contrast trichoviridin has absorption bands at 3580 and 3400 cm^{-1}. In the ^{1}H n.m.r. spectrum of trichoviridin the hydrogen on the carbon atom bearing the secondary hydroxyl group resonated at δ_H 3.76, but in the ester this quartet was shifted downfield to δ_H 5.11; the chemical shifts of all other hydrogens remaining approximately the same. It follows as would be expected from the kinetic data reported by Neises and Steglich (1978) that only the secondary hydroxyl group in trichoviridin had been esterified; another example of the remarkable selectivity of this reagent (Grove et al., 1988). This veratryl ester was recrystallized to constant specific radioactivity (1.82 μCi mmol^{-1}) and it can therefore be concluded that trichoviridin is a degradation product of L-tyrosine.

Among other C_8 isocyanides produced by *T. hamatum* growing on defined salts and glucose media are 1-(2,3-epoxy-1-hydroxy-3-isocyanocyclopent-4-enyl)ethanol (**3**) and isonitrin A (**4**, Fujiwara et al., 1982). The former (**3**) was characterized as its η^5-ethyltetramethylcyclopentadienyl)-di(thiocyanato)-rhodium complex (Boyd et al., 1991) but the catabolite was not purified to constant specific radioactivity. The sample used for preparing the complex was radioactive (2.4x10^4 d min^{-1} mg^{-1}). Fractions containing 'isonitrin A' (**4**) were also radioactive (2.7x10^4 d min^{-1} mg^{-1}) but we remain unable to thoroughly characterize this material (Boyd et al., 1991). If the radioactivities found for **3** and **4** are associated with these compounds rather than with accompanying minor impurities, it would appear that they are also formed by degradation of L-tyrosine.

Experimental

Details of instruments used for spectroscopic measurements and of the purification of reagents are given by McAlees et al. (1990). All extractions were done using a vibromixer in an efficient ventilated space. Effluent air from the fermentor was vented through an incinerator and a stack.

Fermentation, harvesting and extraction of cultures of Trichoderma hamatum - The organism was maintained and inocula grown as previously described (Brewer et al., 1982). The production medium (10 L) containing glucose (400 g), potassium nitrate (20 g) and salts (Brewer et al., 1982) was transferred to a glass fermentor (capacity 15 L) which was stirred at 240 r min^{-1} and sparged with air (4 L min^{-1}) at 25°. The fermentor was inoculated with a culture (500 mL) 48 h old and the pH of the fermentation adjusted to 6.0. The fermentation conditions (volume, pH, air flow, temperature and viscosity) were controlled by the system described by Brewer and his coworkers (1987). After 24 h growth the culture was treated with a solution (2.5 mL) of U-^{14}C-L-tyrosine (203 μCi, 88 mg) in hydrochloric acid (*N*). Forty eight hours after addition of the tyrosine the pH of the culture was adjusted to 8.5 and the fermentation broth separated by filtration. The filtrate was cooled to 4° and then acidified to pH 4.5, when it was extracted (3 x 3L) with ethyl acetate precooled to -15° The ethyl acetate emulsion was broken by filtration through a pad of celite 535 and was then extracted with phosphate buffer (M/15, pH 8.5, 3 x 2L). 3-(3-isoCyanocyclopent-2-enylidene-)propionic acid (**2**) was recovered from the buffer solution as described (Brewer et al., 1982). The ethyl acetate raffinate was concentrated to 3 L, the concentrate kept for 24 h at -15°, when the ice was separated and the filtrate evaporated at <5° to 200 mL.

Chromatography of the neutral ethyl acetate soluble isocyanides - Half of the ethyl acetate solution described in the preceding paragraph (100 mL) was evaporated, the residue dissolved in diethyl ether (50 mL) and petroleum ether (b. p. 30-60°, 8 mL) added. The slightly cloudy solution was applied to the top of a silica gel (80 g, Merck 'for thin layer chromatography') column (4.5 x 12 cm) packed at 4° overnight with the solvent diethyl ether-petroleum ether-acetic acid (300:99:1). The loaded column was developed and eluted with the latter solvent (500 mL), then with diethyl ether-petroleum ether-acetic acid (400:99:1, 250 mL), then diethyl ether-acetic acid (499:1, 250 mL) and finally ethyl acetate (250 mL). The following fractions were collected after application of the sample: 1-6 (each 100 mL), 7-18 (each 50 mL) and 19 (250 mL). Analytical thin layer chromatography (tlc) showed that fractions 4 and 5 contained trichoviridin (3.8 x 10^6 d min^{-1}) and 6,7 and 8 mostly 1-(2,3-epoxy-1-hydroxy-3-isocyanocyclopent-4-enyl)ethanol (**3**, 1.6 x 10^6 d min^{-1}). A third radioactive component (?**4**, 1.2 x 10^6 d min^{-1}) was eluted in fractions 9-11. The second half of the ethyl acetate extract was processed in the same way. The crystalline material from fractions 4 of both chromatograms were combined and dissolved in ether (4 mL), the solution cooled to 0° and hexane (3 mL) layered onto the surface. After 24 h at 4° clumps of colorless crystals (50 mg, 1.9 μCi mmol^{-1}) separated. They were collected and recrystallized from ether-hexane (1:2) giving trichoviridin (45 mg, m. p. 92°, $[\alpha]_D^{24}$ -41° (c, 0.6, $CHCl_3$) 1.82 μCi mmol^{-1}). Fractions 6-11 inclusive (from two chromatograms) were combined and applied as described above to a silica gel (150 g) column prepared with diethyl ether-petroleum ether (1:1). The column (diameter 5.08 cm) was eluted with diethyl ether-petroleum ether mixtures as follows: 1:1, 200 mL; 3:2, 250 mL; 3:1, 250 mL, 4:1, 500 mL and then ether (1 L). The following fractions were collected: 1, (500 mL); 2-6 (each 100 mL); 7-18 (each 50 mL) and 19 (~500 mL). The crystalline material in fraction 15 (16.2 mg, (2.4 x 10^4 d min^{-1} mg^{-1})), was treated in dichloromethane with bis(η^5-ethyltetramethylcyclopentadienyl)di(dithiocyanato)di(μ-thiocyanato)dirhodium(III) (McAlees et al., 1990, 25 mg), the solution kept 3 h at 4°, evaporated and the complex thus formed purified by chromatography on preparative thin layer plates (silica gel, 0.1 x 20 x 40 cm). The complex separated from methyl *t*-butylether as glistening yellow plates (35 mg, m. p. 126°) whose infrared spectrum was identical to that of the complex of 1-(2,3-epoxy-1-hydroxy-3-isocyanocyclopent-4-enyl)ethanol (**3**, Boyd et al., 1991). Tlc (silica gel, solvent diethyl ether) of fraction 19 indicated that it contained a single isocyanide that was radioactive (2.7 x 10^4 d min^{-1} mg^{-1}). It was converted into its $Rh(\eta^5\text{-}C_5EtMe_4)(SCN)_2$ complex m. p. 133° as described (Boyd et al., 1991).

Veratryl trichoviridinate - Trichoviridin (**1**, 152 mg) was dissolved in dichloromethane (3 mL) and veratric acid (149 mg) added. A solution (6 mL) of dicyclohexylcarbodiimide (170 mg) and 4-dimethylaminopyridine (20 mg) in dichloromethane was added to the stirred and cooled (-15°) reaction mixture. After 24 h at -15° a solution (4 mL) of dicyclohexylcarbodiimide (85 mg) was added and the reaction mixture stirred for a further 48 h when the urea (142 mg, 77%) was collected, washed with ice-cold dichloromethane (3 x 2 mL) and the colorless filtrate concentrated to 5 mL. This solution was applied to a silica gel column (4.5 x 15 cm) which was made up and eluted with diethyl ether. Fractions (~ 15 mL) were collected immediately after application of the sample to the column. Fractions 13-16 inclusive contained a single component with greater R_F (ether-petroleum ether, 9:1, silica gel, 0.56) than trichoviridin (0.15). The fractions were combined, evaporated and the colorless crystalline residue recrystallised from carbon tetrachloride giving needles (110 mg, decomp. >50°, m/z 347; υ_{max} (CCl_4) 3560, 2134, 1725, 1720 cm^{-1}; δ_H (CCl_4-$CHCl_3$, 9:1) 7.59, (*J* 8.5 Hz, *J* 1.8 Hz), 7.42 (*J* 1.8 Hz), 6.77(*J* 8.5 Hz), 5.11 (*J* 6.43 Hz), 4.03, 3.86 (6H), 3.44 (m), 3.32 (m), 2.51, 1.44 (3H, *J* 6.43 Hz), 1.82 μCi $mmol^{-1}$). The 4-methoxybenzoyl ester (136 mg, 80%) was obtained in the same way from 4-methoxybenzoic acid (80 mg) and trichoviridin (97.2 mg) as a colorless oil (R_F 0.52; trichoviridin 0.21, ether-petroleum ether 1:1; υ_{max} (CCl_4) 3580, 2131, 1725, 1340, 1165 cm^{-1}; δ_H (CCl_4-$CHCl_3$ 9:1) 7.97 (2H, *J* 9.5 Hz, *J* 2.6 Hz), 6.87 (2H), 5.16 (*J* 6.5 Hz), 4.07 (*J* 2.5 Hz), 3.86 (3H), 3.47, 3.37 (*J* 2.5 Hz), 2.52, 1.48 (3H, *J* 6.5 Hz).

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THE SYNTHESIS OF 1,2-BIS-(4'-(TRIAZENYL)PHENYL)ETHANES[*]

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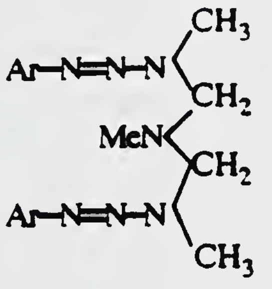
A series of 1,2-bis-(4'-(triazenyl)phenyl)ethanes (**2**) has been synthesised by bis-diazotization of ethylene dianiline, and coupling of the resulting bis-diazonium salt with appropriate alkyl or dialkylamines. The bis-triazenes have been characterized by infrared (ir) and nuclear magnetic resonance spectroscopy (nmr), mass spectrometry and elemental analysis. Line broadening in the nmr spectra of the bis-triazenes is attributed to a combination of conformational equilibria and hindered rotation around the N^1-N^2 triazene bond and also around the C-C bond of the ethylene bridge.

On a synthétisé une série des éthanes de 1,2-bis-(4-(trazenyl)phényl) (**2**) par la bis-diazotisation de l'éthylène dianiline et l'accouplement du sel de bis-diazonium avec des alkyl- ou des dialkyl-amines. On a caractérisé les bis-triazènes par la spectroscopie infrarouge, par la spectroscopie nucléaire magnétique, par la spectroscopie de masse, et par l'analyse élémentale. On attribue l'élargissement des lignes des spectra (rnm) des bis-triazènes à la combinaison d'équilibres conformationnelles autour de la rotation retardée de la liaison N-N et aussi autour de la liaison C-C du pont d'éthylène.

Introduction

Triazenes, also know as diazoamino compounds, are molecules containing three contiguous nitrogen atoms, connected by one double and one single bond. 1-Aryl-3,3-dialkyltriazenes ($Ar-N=N-NR_2$) have been recognised as antitumour agents for some time (Vaughan, 1990) and several compounds of this type are currently in use for treatment of cancer (Lee, et al, 1994). A novel example of a 3,3-dialkyltriazene is the bis-triazene represented by the structure **1**; such compounds are formed as byproducts during the synthesis of 3-hydroxymethyltriazenes ($Ar-N=N-(CH_3)-CH_2OH$) by the reaction of diazonium ions with formaldehyde and methylamine (Manning, et al., 1984).



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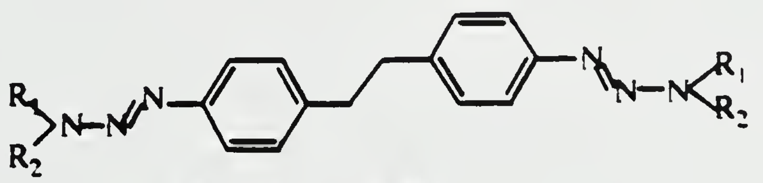
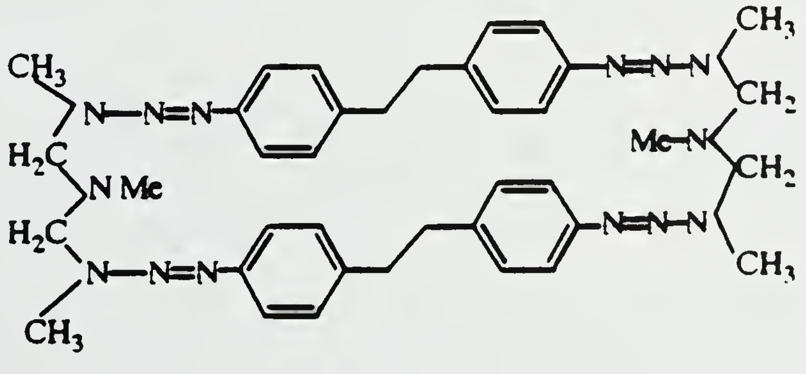


2a R_1=H, R_2=Me
2b R_1=R_2=Me
2c R_1=R_2=Et
2d R_1=Me, R_2=Bz
2e R_1, R_2 = $-(CH_2)_5-$



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A different mode of bis-triazene connection is that represented by the group of compounds **2**, and 1,2-bis-(4'-(3,3-dimethyltriazen-1-yl)phenyl)ethane (**2b**) has been reported to have cytostatic properties (Foerster and Steinhoff, 1970).

A predominant topic in chemistry in recent years has been the discovery and exploitation of macrocyclic molecules, such as the crown ethers (Gokel and Durst, 1976). An interesting extension of this chemistry would be the synthesis of cyclic tetrakistriazenes of the type **3**. Such compounds have not yet been reported and as a stepping stone to the synthesis of such compounds we have undertaken the study of the synthesis and properties of intermediates like **2a**.

Experimental

All reagents were reagent grade materials and were used without further purification. Melting points were determined on a Reichert hot-stage microscope and are uncorrected. Infrared (ir) spectra were obtained using Nujol mulls, on a Perkin-Elmer 299 spectrophotometer. ^1H and ^{13}C nuclear magnetic resonance (nmr) spectra were obtained on C^2HCl_3 solutions at 20° using a Bruker 250 MHz spectrophotometer. Chemical shifts are reported in ppm downfield from the signal of tetramethylsilane. Electron impact mass spectra of compounds **2c** and **2d** were obtained on a Finnigan quadrupolar 4500 machine with a temperature-programmed solids probe. The mass spectrum of compound **2a** was obtained by chemical ionization (isobutane) on a DuPont 21-100 mass spectrometer. Mass spectral data are reported with m/z values followed by the relative abundances in parentheses.

General procedure for preparation of 1,2-bis-(4'-(triazenyl)phenyl)ethanes (**2a-e**) 4,4'-Ethylenedianiline (1.4 g, 0.007 mole) was dissolved in a hot premixed solution of concentrated hydrochloric acid (4 mL) and distilled water (25 mL). The solution was allowed to cool to room temperature and was then filtered to remove insoluble impurities. The ethylene dianiline solution was treated at 0° with a saturated solution of sodium nitrite (1 g, 0.014 mole) over a period of 30 min. The resulting solution was then treated dropwise over 10 min with methylamine or a secondary amine (0.02 mole). After stirring 1 h at 0° a saturated solution of sodium bicarbonate was added dropwise until the reaction mixture was alkaline. The mixture was then stirred for 2 h at 0° when the triazene precipitated from the reaction mixture. The triazene was filtered, dried and recrystallized from hexanes unless stated otherwise.

1,2-bis-(4'-(3-Methyltriazen-1-yl-)phenyl-)ethane (**2a**) - Yield 57%; m. p. ≈230°; m/z 254 (58%), $[M+H-MeN_2]^+$, 240 (84%), $[M+H-CH_3N_3]^+$, 198 (100%); $\nu_{max.}$ 3400-3100 (NH), 830 (*p*-disub.) cm^{-1}; δ_H 3.00 (4H, $ArCH_2$), 3.81 (6H, s, NMe), 7.21, 7.24, 7.61, 7.64 (8H, AA'BB', arom.); δ_C 28.36 ($N\underline{C}H_3$), 37.56 ($\underline{C}H_2Ar$), 115.28, 122.11, 125,84, 129.29 (arom.).

1,2-bis-(4'-(3,3-Dimethyltrazen-1-yl-)phenyl-)ethane (**2b**) - Yield 80%, m. p. 152-154°; (Found: C, 66.9; H, 7.4; N, 25.8. Calc. for $C_{18}H_{24}N_6$ C, 66.7; H, 7.4; N, 25.9%); $\nu_{max.}$ 810, 830 (*p*-disub.) cm^{-1}; δ_H 2.89 (4H, $ArCH_2$), 3.32 (12H, NMe), 7.10, 7.13, 7.31, 7.34 (8H, AA'BB', arom.); δ_C 37.69 ($\underline{C}H_2Ar$), 41.0 (br,$N\underline{C}H_3$), 120.38, 128.98, 139.07, 149.04 (arom.).

1,2-bis-(4'-(3,3-Diethyltriazen-1-yl-)phenyl-)ethane (**2c**) - Yield 79%, m. p. 76-77°; (Found: C, 69.4; H, 8.3; N, 21.9. $C_{22}H_{32}N_6$ requires C, 69.5; H, 8.4; N, 22.1%); $\nu_{max.}$ 840 (*p*-disub.) cm^{-1}; δ_H 1.27 (12H, ^3J 7.17 Hz, Me), 2.91 (4H, CH_2Ar), 3.76 (8H, ^3J 7.17 Hz, NCH_2), 7.10, 7.13, 7.30, 7.34 (8H, AA'BB', arom.); δ_C 13.02 ($\underline{C}H_3$), 37.75 ($\underline{C}H_2Ar$), 44.66 ($N\underline{C}H_2$), 120.32, 128.93, 138.80, 149.39 (arom.); m/z 380 (86) M^+, 308 (28) $[M-Et_2N]^+$, 280 (66) $[M-Et_2N_3]^+$, 208 (72) $[M-Et_2N-Et_2N_3]^+$, 190 (97), 180 (100), 165 (82), 118 (14), 90 (87), 76 (20), 72 (79).

1,2-bis-(4'-(3-benzyl-3-methyltriazene-1-yl-)phenyl-)ethane (**2d**) - Yield 91%, m. p. 117-118°, hexanes-ethyl acetate, (Found: C, 75.4; H, 6.75; N, 17.6. $C_{30}H_{32}N_6$ requires C, 75.6; H, 6.7; N, 17.65%); $\nu_{max.}$ 820 (*p*-disub.), 730, 690 (monsub.) cm^{-1}; δ_H 2.91 (4H, CH_2Ar), 3.12 (6H, NMe), 4.92 (4H, NCH_2), 7.40-7.19 (18H, arom.); δ_C 34.76 ($N\underline{C}H_3$), 37.72 ($\underline{C}H_2Ar$), 58.97 ($Ph\underline{C}H_2$), 120.61, 128.99, 139.31, 148.96 (*p*-disub.), 127.65, 127.67, 128.73, 136.96 (Ph); m/z 476 (55) M^+, 356 (23) $[M-MeBzN]^+$, 328 (10) $[M-(MeBz)_2N_3]^+$, 238 (92) $[MeBzN_3C_7H_6]^+$ 208 (22), 180 (98), 165 (71), 120 (98), 91 (100) $[C_7H_7]$, 90 (85).

1,2-bis-(4'-(3,3-pentamethylenetriazen-1-yl-)phenyl-)ethane - (**2e**) - Yield 87%, m. p. 155-157°, $\nu_{max.}$ 840 (*p*-disub.) cm^{-1}; δ_H 1.68 (12H), 2.91 (4H, CH_2Ar), 3.74 (8H, NCH_2), 7.09, 7.13, 7.32, 7.35 (8H, AA'BB', arom.); δ_C 25.28, 24.45 ($\underline{C}H_2$), 38.0 ($\underline{C}H_2Ar$), 44.51 ($N\underline{C}H_2$), 120.39, 128.51, 128.97 and 139.5 (arom.).

Results and Discussion

Aqueous bis-diazotization of 4,4'-ethylenedianiline followed by reaction with two equivalents of methylamine or a dialkylamine led to the production of 1,2-bis-(4'-(triazen-1-yl-)phenyl)ethanes (**2a-e**) in 57-91% yields. The dialkyltriazenes (**2b-e**) were obtained pure by recrystallization and **2b-d** gave satisfactory elemental analyses. The monoalkyltriazene (**2a**) was not recrystallized but good ir and nmr spectra of this compound were obtained. The ir spectra of all of these compounds showed absorption in the range 810-841 cm^{-1}, as expected, due to out-of-plane bending vibrations of a *para*-disubstituted benzene derivative. Compound **2d** also had additional absorption bands at 690 and 730 cm^{-1} due to the presence of a benzyl moiety.

All of the triazenes showed characteristic AA'BB' couplings in their ^1H nmr spectra with $^3J_{HH} \approx 8.4$Hz (**2a** = 8.55Hz; **2d** = 8.24Hz). The methylene protons of the ethylene bridge fortuitously appeared (in $CHCl_3$) as a singlet, $\delta_H \approx 2.9$ in all the cases examined. The triazene **2b** had predictable N-Me resonance at δ_H 3.32, broadened by hindered rotation around the N^2-N^3 bond (Akhtar, et al., 1968), whereas the NMe resonance of **2a** appeared at δ_H 3.81, probably due to tautomerism (Hooper and Vaughan, 1981). Such tautomerism probably accounts for the difficulty experienced in detecting the signal of the NH proton in **2a**. The ^1H nmr spectrum of the piperidine derivative **2e** was, as expected complex and the multiple couplings of the ring CH_2 groups were not determined. However, the broad resonance at δ_H 3.74 was assigned to the N-CH_2 moieties and that centered on δ_H 1.68 to the remaining ring CH_2 groups.

Further confirmation of the structures assigned to the triazenes **2a-e** was provided by ^{13}C nmr data and assignments are given in the experimental section of this paper. Signals for the bridging ethylene carbon atoms were found in the region δ_C 25.3-44.6 and in some cases these were broadened considerably, possibly due to hindered rotation about the C-C bond.

The electron impact mass spectra of the triazenes **2a**, **2c** and **2d** could be interpreted in terms of the structures proposed for these compounds, though no molecular ion was found in the spectrum of **2a**. Abundant molecular ions at m/z 380 and 476 were observed in the mass spectra of **2c** and **2d** respectively and the further fragmentations of these ions were similar. Both compounds showed the following fragmentation reactions: cleavage of the N^2-N^3 bond, the N^1-aryl bond and the bridging C-C bond.

Fragmentation of both triazene units was also observed and gave the ions common to both spectra at m/z 208 and 180. Chemical ionization mass spectroscopy, of compound **2a** using isobutane as the ionizing gas, did not provide the expected $[M+H]^+$ ion at m/z 297 but did produce an abundant ion at m/z 254 possibly arising by protonation of N^1 and subsequent fission of the N^1-N^2 bond. Another abundant ion at m/z 240 probably involves loss of the fragment CH_3N_3

The experiments described show that bis-triazenes of type **2** can be prepared in good yield and that their physical and spectral characteristics are consistent with those oF known simpler triazenes. This knowledge will be useful in the endeavor to synthesize macrocyclic tetrakis-triazenes e.g. **3**.

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A FIRST PRINCIPLES APPROACH TO THERMAL CONDUCTIVITY MEASUREMENTS OF SOLIDS*

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Although knowledge of heat conduction is crucial to the understanding of many practical everyday phenomena, the exposure of university students at the undergraduate level to this subject is often limited. With the intent of improving this situation, we describe the design of a simple apparatus for measurement of the thermal conductivity of materials, using components that could be found in most undergraduate laboratories. Use of this apparatus clearly illustrates the basic principles of heat conduction, as the data collected enable the determination of the thermal conductivity of a material directly from the defining equation. To demonstrate the use of this apparatus in determining the thermal conductivity of materials, we describe the result obtained from measurements of a plate of aluminium alloy.

Bien que la connaissance de la conduction de la chaleur soit critique à la compréhension de plusieurs phénomènes pratiques journaliers, l'exposition de ce sujet aux étudiants universitaires au niveau non-diplômé est souvent restreinte. En vue d'améliorer cette situation nous décrivons le plan d'un simple appareil qui mesurerait la conductivité thermale de matériaux, avec les composants que l'on trouve dans la plupart des laboratoires. L'usage de cet appareil démontre clairement les principes fondamentaux de la conduction de la chaleur, puisque les données qu'on relève permettent la détermination de la conduction thermale du matériau soumis à l'examen directement de l'équation définissante. Afin de démontrer l'usage de cet appareil, nous décrivons aussi le résultat obtenu des mesures d'une plaque d'alliage d'aluminium.

Introduction

Thermal conductivity is a fundamental and important property of matter. As the name implies, the thermal conductivity of a substance is a quantitative measure of how perfectly the substance conducts heat. Thermal conductivity values for different materials can vary over several orders of magnitude, and this fact has many practical applications in everyday life. For example, the high thermal conductivity of metals such as iron and copper make these substances useful in the construction of pots and pans for cooking, since heat will be distributed evenly throughout the metal and food will cook uniformly. However, coffee mugs are normally made from low thermal conductivity ceramic materials to minimize the heat exchange between the coffee in the mug and its surroundings, to keep the coffee warm.

Despite its fundamental and practical importance, the concept of thermal conductivity usually receives only brief mention in undergraduate chemistry classes. Although the basic concept can be introduced with the kinetic theory of gases, a thorough study of heat conduction in materials would require a working knowledge of the detailed theories of solid-state physics and chemistry which are normally beyond the scope of undergraduate courses. Experiments illustrating the basic principles of thermal conductivity measurements could introduce students to the idea of thermal conductivity at an undergraduate level, and an experimental determination of the thermal conductivity of gases in the undergraduate laboratory has been published (Shoemaker and Garland 1967). The wide range of thermal conductivities of technologically important materials would make a simple experimental method for determination of thermal conductivities of solids a useful addition to the undergraduate physics or physical chemistry laboratory.

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Very accurate thermal conductivity measurements require that the system of interest be thermally isolated to prevent unwanted heat exchange between the system and the surroundings. Such measurements would require the design and construction of a sophisticated piece of apparatus, and typical methods have been summarized (Berman 1976; Touloukian 1970). However, our concern in the development of the present experiment was not high accuracy, but rather to design a simple experimental procedure that would allow a good estimate of the thermal conductivity of a material.

Experimental Method

Thermal conductivity (κ) is a transport property that is defined by the following equation:

$$\dot{q} = -\kappa(dT/dx) \tag{1}$$

where $\dot{q}$ is the heat flux through the material in the measured direction (represented as the x-direction in this case) and dT/dx is the corresponding temperature gradient. Heat flux is the rate of heat flow per unit area (*i.e.* $\dot{q} = \dot{Q}/A$ where $\dot{Q}$ is the rate of heat flow and A is the cross-sectional area). There are many other equations describing thermal conductivity which are derived from equation 1. However, our approach to thermal conductivity measurements uses the definition (equation 1) directly.

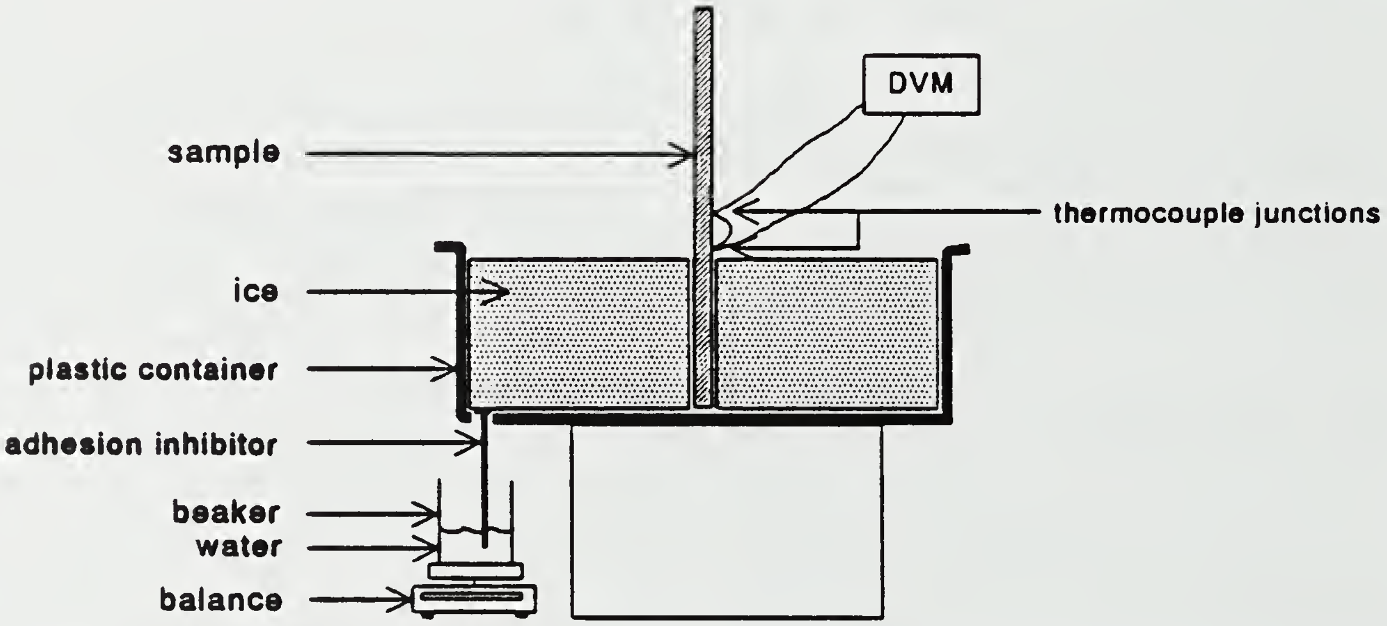

Fig 1 A schematic diagram showing the experimental set-up that was designed and used to measure the thermal conductivity of a plate of aluminium alloy. Dimensions are given in the text. DVM = digital voltmeter.

The experimental apparatus that was designed and used in this study is shown schematically in Fig 1. The basis of the experiment was the determination of the temperature gradient across a sample during conduction of heat from the room, through the sample, to melt ice in which the sample was imbedded. (The use of melting ice in thermodynamic determinations has a distinguished history, starting with the ice calorimeter designed by Lavoisier and LaPlace (1780)). The heat flux was determined by the rate of melting of ice (relative to the rate in the absence of the conducting material), and the thermal conductivity, κ, was determined from the direct use of equation 1.

The apparatus consisted of a rigid plastic container (a dish pan, 35 x 25 x 12 cm deep was used; better results were obtained when the pan was insulated) with a ~0.5 cm diameter hole in one corner. The material to be measured (in the form of a plate) was placed vertically in the centre of the plastic container, held in place by the ice which filled the plastic container. As the ice melted, the resulting water flowed out of the hole in the plastic container into a beaker which was resting on an analytical balance (0.1 g precision) below the pan. The rate of water production might be determined by volume, but this would need both 0.1 mL precision and calibration at the temperature of the water. A narrow piece of plastic tubing (~1 mm OD) was placed between the hole and the beaker to act as an "adhesion inhibitor" so that there was a steady flow of water between the plastic container and the beaker rather than an uneven dropwise flow; without the "adhesion inhibitor" mass measurements were uncertain. Once a steady flow of water was achieved, the mass of water in the beaker as a function of time was measured. The water mass increase rate (dm/dt) can be converted to rate of heat flow ($\dot{Q}$=dQ/dt) by multiplying by the enthalpy of fusion of water ($\Delta_{fus}H = 333.7\ \mathrm{J\ g^{-1}}$ (Adamson 1979)), i.e.

$$\dot{Q} = dQ/dt = \Delta_{fus}H(dm/dt). \tag{2}$$

There are two heat channels which cause melting of the ice in this configuration: (1) heat flow through the material being measured, and (2) heat flow through the remaining parts of the set-up. The measured increase in mass of water (which corresponds to the rate of heat flow, $\dot{Q}$) is made up of the contributions from both of these sources. To compensate for the melting of ice that would take place in the absence of the material being measured, mass flow measurements were initially taken without the material in the container. After steady-flow conditions were achieved (this took about 15 minutes), the mass of water was determined as a function of time (typically every 30s, for about 10 minutes). Then the material to be measured was placed in the container, a waiting time (*ca.* 15 minutes) for steady-state conditions was allowed, and mass measurements were again taken as a function of time. The difference between dm/dt with and without the sample, is the rate of mass change due to heat flow through the material, dm/dt of equation 2.

The cross-sectional area of the material, A, was needed in order to calculate the heat flux through the material, $\dot{q}$. This value was determined using a ruler and a vernier calliper.

Measurement of the temperature gradient along the face of the material was carried out using a thermocouple. One junction of a 0.08-mm diameter AuFe (0.03 atom % Fe)/Chromel (Johnson Matthey; supplied and calibrated by Cryogenic Calibrations Ltd., HP22 4HT United Kingdom) differential thermocouple was attached to the sample close to the ice, to minimize the effects of lateral heat flow from the surroundings. The other junction was placed vertically above the first such that there was a reasonable distance (5-10 cm) between the two. The thermocouple junctions were covered with a thin layer of silicone grease (necessary for good thermal contact) and held in place with adhesive tape. The distance between the junctions was determined with a ruler. A fine-gauge AuFe/chromel thermocouple was used because of its known high accuracy and the low thermal conductivity along the thermocouple itself. Other thermocouples (*e.g.* copper-constantan or chromel-alumel) could function well for this set-up, but fine gauge wires should be chosen in order to make the heat flux through the sample dominate over that through the thermocouple wire. During the series of water mass measurements, the potential difference between the two thermocouple junctions was constantly monitored with a digital voltmeter until a steady-state value was reached and this value was recorded. This voltage was later converted to a temperature difference using a calibration table for the AuFe/chromel thermocouple (Cryogenic Calibrations Ltd.).

A plate of aluminium alloy (≈ 350 mm x 200 mm x 3 mm) was measured as a test of the apparatus. An analysis of the plate by an electron microprobe with an energy-dispersive detector gave the following results: 97±1 atom% Al; 2.5±0.1 atom% Mg; 0.2±0.1 atom% Cr; 0.15±0.05 atom% Si; 0.15±0.01 atom% Fe.

Results and Discussion

Plots showing the mass of water flowing into the beaker as a function of time before and after insertion of the sample are shown in Fig 2. Both plots are linear and the slopes indicated on the graphs were obtained by linear least-squares fit. These slopes correspond to the water mass increase rate, and the difference between them (dm/dt after insertion of plate - dm/dt before insertion) gives dm/dt due to the heat flow through the plate. The thermal conductivity, κ, was calculated using equation 1. For the aluminium plate measured, $\kappa = 120 \pm 20$ $W.m^{-1}.K^{-1}$ at T = 280K. The error range reflects the standard deviation on five measurements. One measurement was carried out with the pan and the ice surface insulated with 2.5 cm thick styrofoam block; the thermal

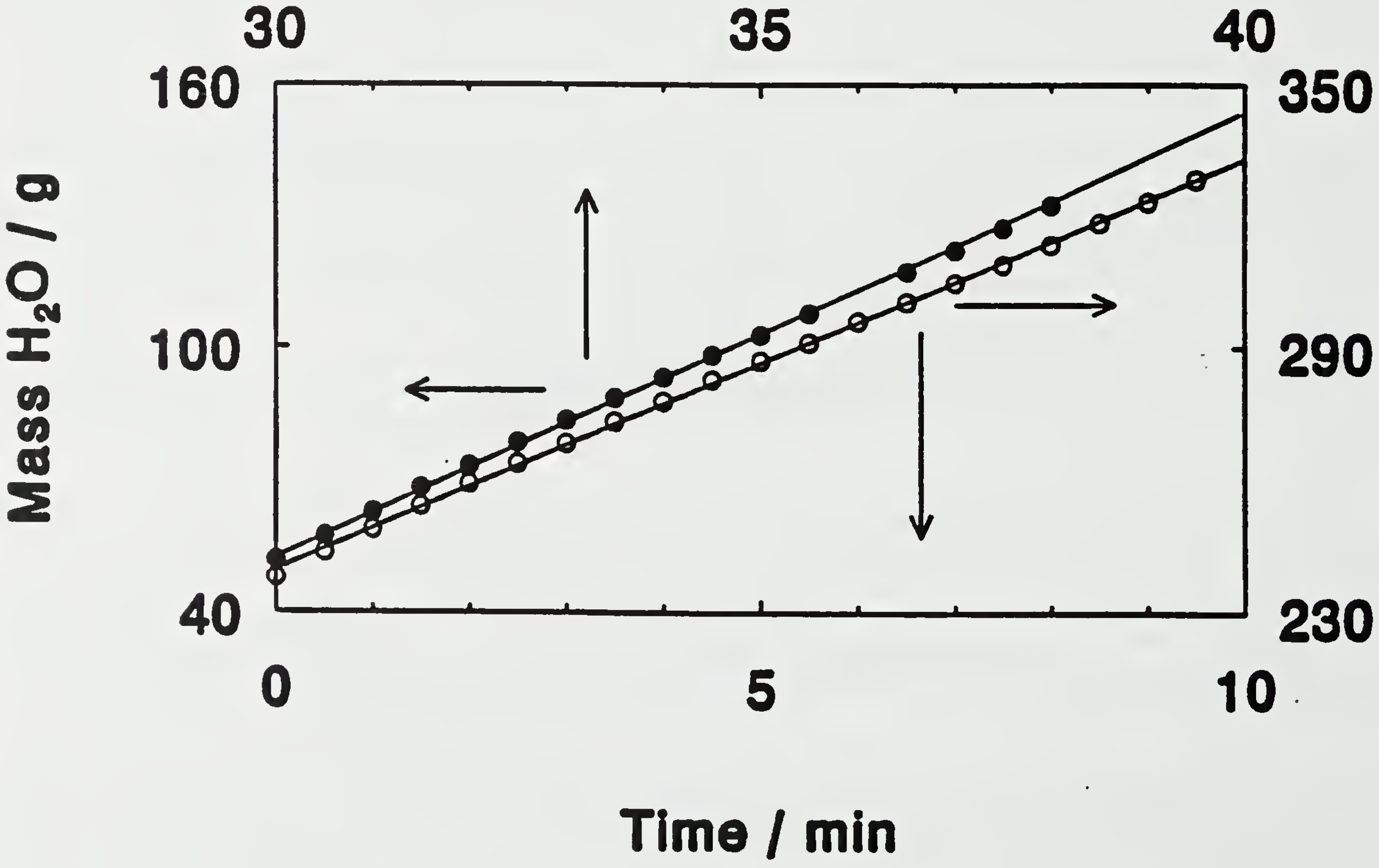

Fig 2 Plots of the measured mass of H_2O in the beaker *versus* time before (o) and after (•) the Al plate was inserted into the apparatus. The slopes, as indicated by the least-squares fit lines, are the rates of mass increase, dm/dt. (The later masses are less than the earlier ones because the beaker was emptied between run ◦ and run •.)

conductivity was essentially the same but the increase in dm/dt was more substantial (≈50% increase with insulation; ≈20% increase without insulation). Since a larger increase in dm/dt will reduce the uncertainty in the determination of the thermal conductivity, insulation is recommended.

This experiment has not been designed to provide the most accurate measurement of thermal conductivity, and there are sources of errors. The main sources of error are the thermal contact between the thermocouple junctions and the plate, and uniformity in water flow rate. Another error is that heat exchange by radiation and conduction between the material and the surroundings in the region between the thermocouple junctions is not taken into account; in principle, this would lead to steeper vertical temperature gradients closer to the ice than further out and the value of dT/dx in equation 1 could be inappropriate if the thermocouple junction separation were so great that lateral heat flux was important. However, we have carried out measurements with various thermocouple junction separations (5-10 cm) and do not find that this correlates with the measured thermal conductivity, showing that equation 1 is appropriate at these separations for this material.

Although the uncertainty in the determination is rather large (±17%), on the basis of measurements of thermal conductivities of Al alloys of similar composition (Touloukian 1970), the thermal conductivity of this sample at about 280K (the mean sample temperature) would be expected to be between 100 and 200 W.m^{-1}.K^{-1}, and the observed value is within this range. When making comparisons with literature values, it should be noted that the presence of even small amounts of impurities can reduce the thermal conductivity of metals significantly (Berman 1976): *e.g.* the thermal conductivity of very pure aluminium at about 280K is 235 W.m^{-1}.K^{-1} (Touloukian 1970).

It is possible to use this method to measure thermal conductivities of other materials. In order to achieve meaningful measurements, the material to be investigated must sufficiently enhance the heat flux to the ice to produce a measurable increase in the rate of melting (compared to the absence of the material), while having a sufficiently large temperature gradient to be measured accurately with a thermocouple (preferably of the order of 1K.cm^{-1}). The dimensions of the sample to be measured can considerably influence these factors.

Other possible aspects that could be explored include the effect of the material's colour (painting the plate black led to an enhanced heat flux), or the material's geometry (κ should be independent of geometry). Given the relevance of thermal conductivity to materials of technological significance, it is likely that students and teaching staff could help the experiment evolve in many different directions.

This experiment can introduce or enhance the following subjects in the undergraduate teaching laboratory: connections between thermodynamics and the "real world"; the use of thermocouples; error propagation; least-squares fitting; discussion of precision *vs.* accuracy of a measurement; quantitative effect of purity on a physical property. Given the simplicity of the experiment, these aims can easily be accomplished within the two- to three-hour timeframe of an undergraduate laboratory period.

Conclusions

This experimental procedure for measuring thermal conductivity is a rudimentary one, and yet leads to measurements of good accuracy. Furthermore, the determination is based on the defining equation for thermal conductivity. The set-up is so simple that it could be used as an instructive undergraduate-level laboratory exercise to introduce students to the concept of thermal conductivity.

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CURVED ARROWS AND THE ELECTRONIC THEORY OF ORGANIC CHEMISTRY*

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The historical genesis of the curved arrow in organic chemistry is described during the debate in the pages of Chemistry and Industry (Review Section) from 1923 to 1926 between Robert Robinson, Christopher Ingold and others. Following Ingold's article in Chemical Reviews in 1934, the gradually increasing use of the curved arrow, first in advanced research level books on physical organic chemistry and then in entry level organic chemistry textbooks, is discussed.

La genèse historique de la flèche courbée en chimie organique est le sujet de débat sur les pages de Chemistry and Industry (Review Section) depuis 1923 jusqu'à 1926 entre Robert Robinson, Christopher Ingold et d'autres. On discute de l'usage progressivement accroissante de la flèche courbée d'abord dans les livres au niveau de recharche en chimie physico-organique et ensuite dans les manuels de chimie organique de première année d'études universitaires.

Introduction

In the preface of a recently published textbook for teaching organic reaction mechanisms (Weeks, 1992), the author remarks, "At the introduction of resonance or mechanisms, the professor begins using little arrows that resemble fish-hooks.* Using these arrows, electrons are moved around molecules and appear - is if by wizardry - precisely where the professor needs them. The penchant for moving electrons has earned organic chemists the pejorative *electron pusher* that .. we wear proudly." In agreement with this statement, the curved arrow has been called "the most important symbol in twentieth-century organic chemistry" (Brock, 1992). In another text (Scudder, 1992), the author warns, "We use the concept of electron flow together with rigorous use of curved arrows as an electron book-keeping device. The use of curved arrows without mechanistic constraint has been rightfully criticized. Because a process can be drawn with arrows has no bearing on its correctness. It often seems that novice "electron pushers" are just trying to rearrange the lines and dots of reactant structure into the lines and dots of product structure with a minimum number of intermediates.... There is much less tendency to write nonsense with arrows if you are assembling proven mechanistic units, the electron flow pathways."

Three examples of the use of these arrows for reaction steps, as they would appear in modern introductory organic chemistry texts, are presented. The tail of the arrow shows where the reactive electron pair is and the head shows where it is going. The curved arrows then generate new structures that appear as product(s).

Equation 1 shows the neutralization of a base (HO^-) by an acid (H_3O^+). In the Brønsted definition, the acid is a proton donor and the base is a proton acceptor. In the Lewis definition, the base is an electron-pair donor and the acid an electron pair acceptor. For the latter definition, electron flow is clearly illustrated by the curved

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* Dedicated to the memory of Walter J. Chute.

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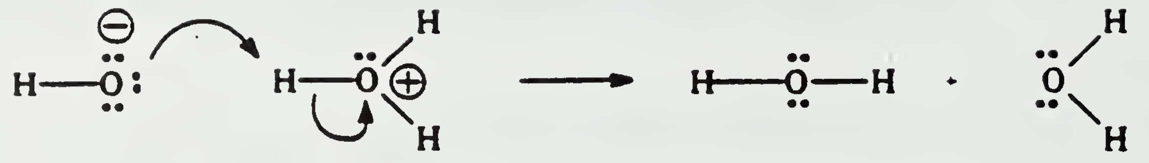
* This term is normally used to indicate homolytic bond cleavage and formation. In this paper double headed arrows represent motion of electron pairs, i.e. heterolytic bond formation and cleavage



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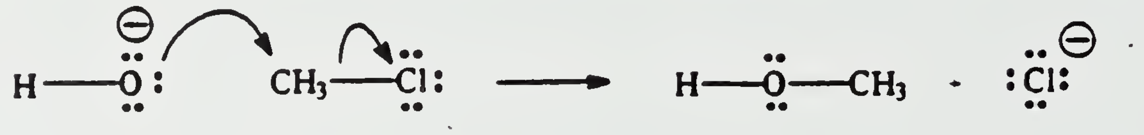

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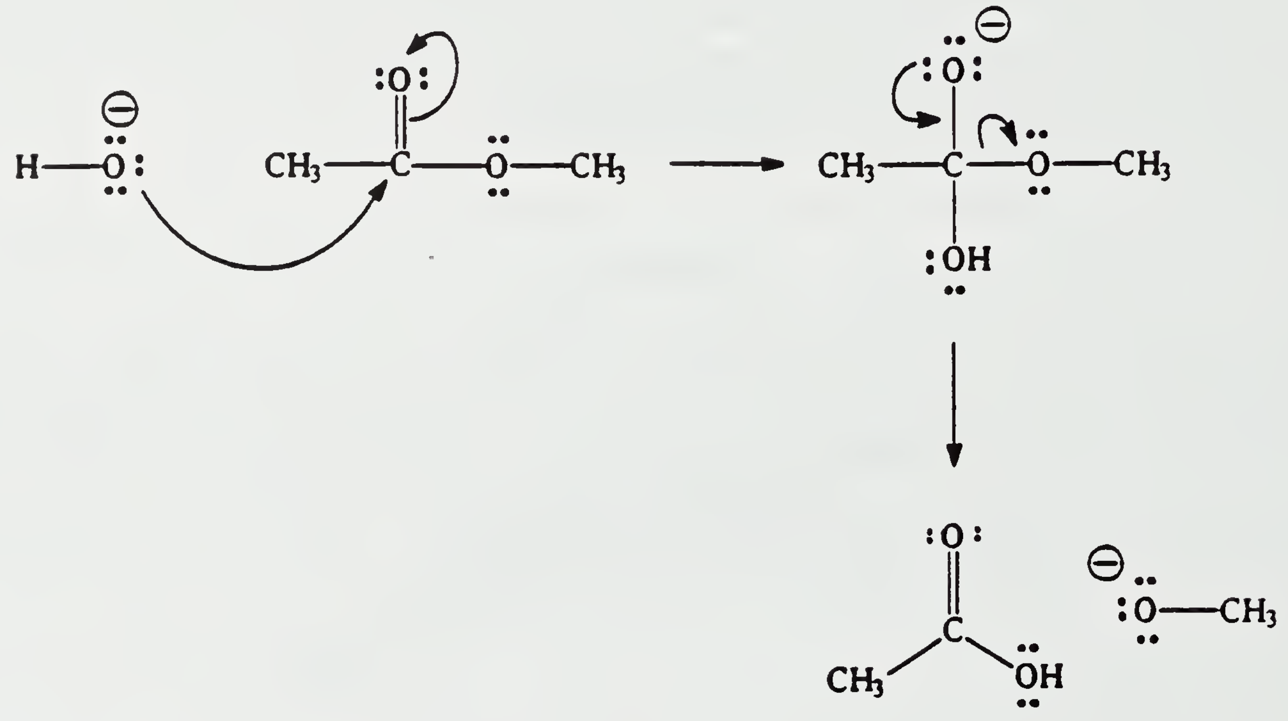

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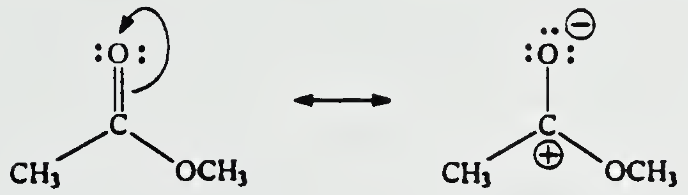
arrows which are book-keepers of the charge. As electron density moves from negative hydroxide ion to positive hydronium ion, the oxygens of both become neutral. Equation 2, an S_N2 substitution, also shows the Lewis base acting as an electron-pair donor; hydroxide ion (HO^-) is now called a nucleophile because it reacts with an electron deficient carbon-centered acid called an electrophile; i.e. an electron pair acceptor or Lewis acid. The saponification of an ester (Equation 3), shows how the curved arrow method can be applied to a two-step reaction. The rate-determining step, reaction of the hydroxide ion (nucleophile) with the carbonyl carbon (electrophile), leads to an intermediate which then rapidly decomposes to the products.

It is often possible to draw more than one resonance structure for a compound or reaction intermediate (a double headed arrow defining contributing resonance forms) and curved arrows can be used to illustrate which electron pairs change position when a new structure is generated. In Equation 4, because oxygen is more electronegative than carbon, there is no ambiguity about the polarity of the bond represented by the charge-separated resonance form. This polarity indicates why the carbon should react with nucleophiles and the oxygen with electrophiles. In Equation 5, the charge-separated form suggests that the methylene group will react with nucleophiles, while Equation 6 infers that it will react with electrophiles. These examples illustrate that although curved arrows can be drawn for two possibilities, one must understand which makes more chemical sense. In this case, styrene reacts rapidly with electrophiles (Equation 6) but not with nucleophiles (Equation 5).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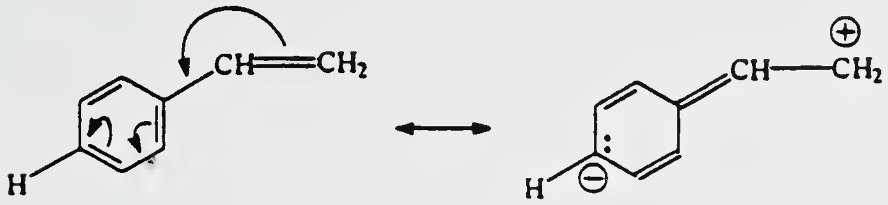
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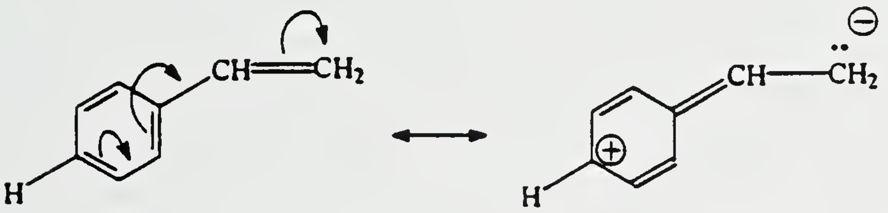
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This interpretation of reactions by the movement of electrons, represented by curved arrows or sometimes more colloquially "curly arrows", is now a ubiquitous part of the thinking of organic chemists, but most practitioners of this field have little knowledge of how these concepts of the electronic theory came to dominate organic chemistry.

The history of the development of the electronic theory of organic reactions

The birth of this electronic theory and the accompanying curved arrow is one of the more colorful episodes in the history of organic chemistry. The theory of organic reactions developed over about three decades, and started just before the beginning of the twentieth century, with two publications by Thiele (1899) in which he postulated "an affinity residue or partial valency". Flürscheim (1902) used these publications and Werner's theory of variable valency to develop his own theory of alternate affinity to explain the *ortho/para* to *meta* ratios of the products of aromatic substitution reactions. He continued the development of his theory for the rest of his career and was never able to accept the concept of the electron pair in covalent bonding and organic reactions. This concept of a chemical bond was introduced by Lewis (1916), but it took several years, and Irving Langmuir, who " was to act as publicist for the shared pair immediately after the First World War" (Brock, 1992, p 477) to impress its importance on the organic chemist.

About 1916, Arthur Lapworth, one of the first chemists to elucidate mechanisms of organic reactions, put forward the theory of alternating polarities. Robert Robinson, who was a colleague of Lapworth's at Manchester University, began using this theory in his interpretation of reactions at about the same time, though Lapworth did not publish his theory until 1920 (Lapworth, 1920). By 1922, Robinson had picked up the electron pair theory of bonding and then he began writing organic reactions in these terms, using a curved arrow to denote the movement of electron pairs in a molecule (Kermack and Robinson, 1922). Arrows had appeared in the earlier literature, but this was the first use made of them to indicate the movement of electrons. These ideas began to appear in advanced texts almost immediately (Cohen, 1923) although a review of them was not encouraging: "This is perhaps the least satisfactory portion of the book, because it is doubtful whether any useful purpose is served by burdening the students' mind with a mass of theoretical and practical detail much of which is non-proven and all of which is highly controversial. Its intrusion at the present time is apt to lead to confusion if only because its many votaries seem incapable of reaching agreement among themselves" (Thorpe, 1923).

No doubt the modern theory of organic reactions would have assumed, more or less, its present form even had the leisurely pace of advance continued. However, it was given a volcanic upheaval in the mid-1920's. This extraordinary episode in the development of the theory of physical organic chemistry was played out at the monthly meetings of the Chemical Society in London, in the reports of those meetings and in the letters to the editor in the review section of *Chemistry and Industry.* Over the course of about three years, the debate led to a transformation of the pre-electronic theories of organic chemistry of Thiele, Flürscheim and Lapworth to the beginnings of the modern view of organic reactions based on Ingold's terminology and concepts.

"It also left Robert Robinson feeling that his contribution to the new theory had been unacknowledged and that Ingold, in fact, had stolen his ideas. He carried this grievance for the rest of his 88 years and was still calling "Stop thief!" from beyond the grave, since he renewed the cry in his autobiography, which was published posthumously, the year following his death. "... these ideas constituted, in the writer's [Robinson] opinion, his most important contribution to knowledge..." (Robinson, 1976). There were many who felt that this grievance caused Robinson, in later years, to block the award of the Nobel Prize to Ingold. There is, however, no evidence that Robinson ever did more than fail to support Ingold's nominations for the prize.

There were five principal players in the debate, the oldest of whom was Lapworth, 51 years old in 1923. Next was T. Martin Lowry, 49 years old and professor of physical chemistry at Cambridge. Both of these men had been taught by H.E. Armstrong in London. Third was Bernard Flürscheim, a chemist of independent means, born in Baden Baden, and who had studied at the University of Geneva, and then worked at Zurich under Werner and at Strasbourg with Thiele. He obtained his Ph.D. in 1901 at Heidelberg and in 1905 settled at Fleet in Hampshire, U.K., where he built his own laboratory. He remained there for the rest of his life, except for a few temporary paid positions and First World War work. The other two protagonists were younger - Robinson, at 37 had just become Professor of Chemistry at Manchester, junior to Lapworth, and Christopher Ingold, 30, was a Lecturer at Imperial College under J.F. Thorpe. Others, such as Thorpe and Boyd, contributed to the debate from time to time, the subject and emphasis of which often changed, making for an involved and confusing series of events. The main players frequently misunderstood and misinterpreted each other, so that other workers, not actually involved but trying to follow the developments, sometimes broke into print themselves to express their exasperation with the principal participants.

The older men were essentially defending their theories. By contrast Robinson, at the peak of his intellectual powers, and Ingold - who had recently won two Meldola medals and who was in a phase of extreme career acceleration - were both willing to change and develop their ideas. In the end it came down to a conflict between these two.

The dispute initially broke out upon the presentation of a paper at the Chemical Society meeting on June 21, 1923 (Baker, Ingold and Thorpe, 1923) which was one of a series on ring-chain tautomerism in the mutarotation of sugars. It presented evidence that there was no hydrated intermediate in this reaction, contrary to the theory held by Lowry. The argument was not resolved but it can be seen with hindsight that Ingold was arguing that the water molecule was not involved in the rate determining step of the reaction, whereas Lowry was arguing that water was involved somewhere in the overall reaction. They were, in fact, both right; the differences expressed were academic and Lowry signed Ingold's nomination proposal for election to the Royal Society.

However, in June 1924 the controversy began in earnest with a paper by Boyd (1924) entitled "Hydrolysis and the theory of induced polarities" which cited results in direct conflict with the theories of Lapworth and Robinson. Robinson responded vigorously, claiming that Boyd had wrongly interpreted his examples. Lowry maintained that there was no justification for the assumption of alternate polarities in the case of saturated compounds. Flürscheim agreed with Boyd, and G.T. Morgan expressed his exasperation with a theory whose proponents continually shifted their ground. Ingold moved to support Boyd's paper "as a much needed protest against *ex cathedra* utterances relating to polarity theory, and the chosen evidence by which it was widely upheld; moreover, the type of criticism employed was very widely applicable". During his discourse, Ingold wrote the formula of styrene as $^{+}\mathrm{Ph.C^{-}H{:}CH_2^{+}}$ (note Equation 5).

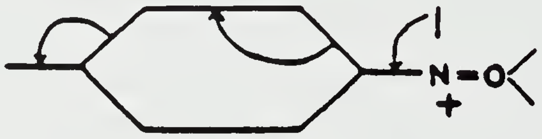
In the July 1924 issue of *Chemistry and Industry*, Lowry published a letter (1924b) objecting to this formula of styrene apparently carrying a positive charge as if it were a univalent cation like NH_4^+. He wrote "There is no need for me to defend the theory of induced alternate polarities, which is quite secure in its Manchester stronghold. My own responsibility has been incurred in suggesting an electronic mechanism, which makes the phenomenon inevitable in conjugated systems undergoing chemical change, but has also the effect (fortunate or unforunate) of limiting it to conjugated systems - except, perhaps, in those cases where a single bond can be ionized or activated in the same way as the double bonds of a conjugated system".

Thus, Lowry demonstrated that he had a clear concept of how the theory of organic reactions was developing. Despite this, his letter triggered vigorous responses. The first came from Lapworth (1924) who claimed that, historically, the + and – signs had been used, "to indicate the *kind* of electrification, and this therefore had priority over the quantitative application. It seems to me undesirable to restrict the significance of the older signs, and the onus of finding new signs with a quantitative meaning such as Professor Lowry requires for his electro-covalent double bonds rests with him". Lapworth's historical argument was, rejected by chemists and the + and – signs were retained only for full charges. Another terminology, $\delta+$ and $\delta-$, was invented by the Ingolds (Christopher and Hilda, his wife) for fractional charges and was introduced two years later (Ingold and Ingold, 1926). In modern presentations, the better texts do not use $\delta+$ and $\delta-$, instead, partial charges are described by resonance contributors (forms) and the charges on those with charge separation are called "formal charges". Thus in Equation 4, the charges on the right hand structure are formal charges on a resonance "form" but the overall charge on the molecule is zero.

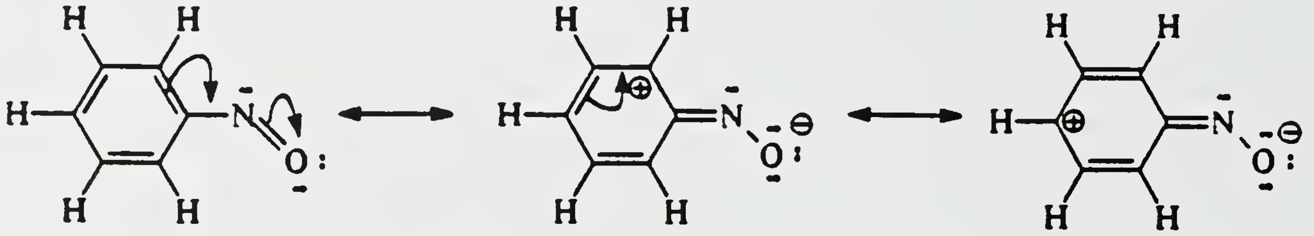
Further letters were exchanged between Lowry and Lapworth into the summer of 1924 and additional contributions to the debate made by Boyd and H.G. Rule, extended into the autumn of that year. Ingold's next statement came on October 16, 1924 in response to a paper presented at the Chemical Society on "The nitration of 2-phenylglyoxime and its carboxylic acids" (Pyman and Stanley, 1924). These authors invoked the theory of induced alternate polarities to explain their results, to which Ingold responded by claiming "equality of consideration for the alternate polarity theory and Flürscheim's theory of variable affinity in cases where the experimental results could be explained equally satisfactorily by the latter as by the former".

This discussion of the mechanism of nitration of 2-phenylglyoxime focused attention on the problem of the orientation of a second substituent when an aromatic compound e.g. chlorobenzene, underwent further substitution. Obviously reaction could occur at either the 2-(*ortho*), 3-(*meta*) or 4-(*para*) position and the challenge was to develop a theory which could predict the relative proportions of the three products for any given mono-substituted benzene.

At the December 18, 1924 meeting of the Chemical Society, Ingold (1924) presented a paper on the "Directing influence of the nitroso group in aromatic substitution", stating that "the results are in better harmony with the view that the alternation along a carbon chain is an alternation in the quantity, rather than in the electropolar quality, of the residual affinity". Robinson was not at this presentation but sent a letter (Robinson 1924) that was read by the Secretary and explained why the nitroso group led to *ortho*/*para* substitution products. This included a diagram using curved arrows to show the flow of electrons in a molecule during a chemical reaction. Although Kermack and Robinson (1922) had used curved arrows two years earlier, this was the first effective use of them in the substitution debate. Robinson wrote, "Representing an electron pair by a line, the following exhibits the covalency change leading to the negatively charged *para* position:



Polar activation and valency redistribution occur simultaneously and are not, as Professor Ingold seems to imply irreconcilable hypotheses".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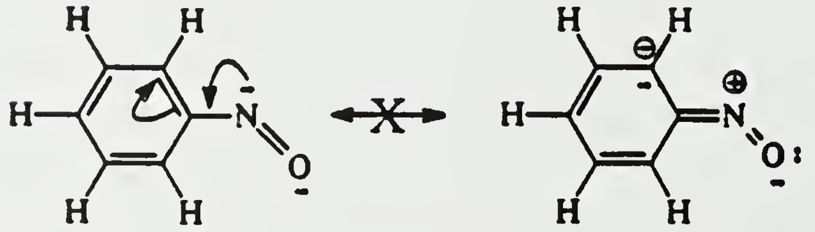
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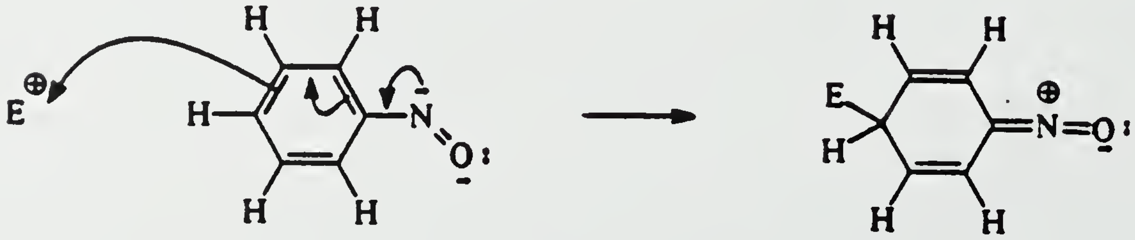
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In retrospect, the nitroso substituent is one for which a prediction of its ability to donate or withdraw electrons by the resonance effect is not obvious. As shown in Equation 7, the nitroso group is electron withdrawing because the π bond of the NO group is conjugated to the π bonds of the aromatic ring. The *ortho* and *para* carbons will be electron deficient. The nitroso group cannot be an electron pair donor because the lone pair on nitrogen is not conjugated to the benzene π bonds but occupies the orthogonal σ plane (Equation 8). Although the nitroso group could possibly stabilize the *ortho* and *para* intermediate (*para* shown in Equation 9) of electrophilic aromatic substitution by changing its geometry from bent (sp^2 hybridized) to linear (sp hybridized), the experimental observations are that the nitroso group is strongly electron withdrawing (Equation 7) and only activates the ring towards nucleophilic aromatic substitution (Boyer, 1969).

Perhaps because Robinson was not at the meeting, his argument did not capture the audience. Professor G.T. Morgan "disclaiming any knowledge of this plus and minus business" was on the whole inclined to prefer Flürscheim's theory. Flürscheim thought that Robinson was "trying to make on the swings what he had lost on the roundabouts". Ingold reacted uncharacteristically by making no effort to rebut Robinson or to win the debate - he merely said "that his paper was primarily intended to publish a new experimental fact; he found great difficulty in understanding the views expressed by Professor Robinson". Ingold was not a person who frequently had difficulty understanding chemical arguments. At this point he quietly relinquished the position of standard bearer for Flürscheim's theory and concluded the discussion by saying that "The issue could only be settled by crucial experiments in some dozens of suitable cases". Thus 1924 closed with relations between Robinson and Ingold still quite harmonious.

In 1925 Ingold was still searching for the definitive experiment. When he thought that he had it he wrote again to *Chemistry and Industry* (Ingold 1925a) pointing out that Robinson had said that $PhCH_2N^+H_3$, $PhCH_2NHCOMe$ and $PhCH_2NEtPh$ would all react to give *meta*-substituted products according to the alternating polarity theory. Ingold had found, experimentally, that the first two gave *ortho*- and *para*-substituted products. The third compound was, as yet, untested. However, he did not repeat his indiscretion of the previous year and claim that the polar theory was wrong but emphasized the triumph of experimental results over debate. "That the facts .. though agreeing with Dr. Flürscheim's theory, are opposed to Professor Robinson's predictions, is perhaps an appropriate answer to Professor Lapworth's contention that it is impossible, by experiments on aromatic substitution, to distinguish between polar and non-polar theories of directive action". But Lapworth replied that "the real question was :- do such experiments prove that any of these directive effects are non-polar? - the only truthful answer is in the negative". Ingold eventually had to concede that point.

At the June 18, 1925 meeting of the Chemical Society, Robinson presented a paper "The relative directing powers of groups of the form RO- and RR'N- in aromatic substitutions. Part IV. A discussion of the observations recorded in Parts I, II and III". The paper (Allan, Oxford, Robinson and Smith, 1926) summarized his experimental results and interpreted them in the light of his most recently developed theory. The theory was attacked by Flürscheim in the discussion but Ingold was a lot more cautious saying, "The value of Professor Robinson's theory as a stimulant to research depended on its freedom from ambiguity and the definiteness of its consequences, matters on which it was difficult to form an opinion until the theory had been published. The principle of starting with an *ortho*- compound and considering only substitution on the other side of the ring ... appeared sound and was being employed at Leeds to compare the directive powers of different atoms". Robinson expressed appreciation of Professor Ingold's remarks.

Ingold was still trying to find the definitive experiment to test the two theories, but this was not possible until a final form of the alternating polarities theory was settled. His exasperation with Robinson's "adjustments" began to show in a letter of June 20, 1925 (Ingold, 1925b) which concluded "[Robinson's] letters make it clear that either the verification or the non-verification of Professor Robinson's predictions would constitute evidence in favour of his theory, and that Professor Lapworth holds his opinions with great tenacity".

On August 14, 1925, the first long letter of the debate from Ingold (Ingold, 1925c) appeared, discussing the relative tendencies of tervalent nitrogen, bivalent oxygen and fluorine to set up alternations which control aromatic substitution. Ingold quoted extensively from Robinson's papers tracing the adjustments which Robinson made to his theory to change the sequence from F>O>N to N>O>F, the experimentally observed result. The letter concludes "The recent 'adjustment' of polarity theories lends support to Professor Lapworth's denial of the possibility of testing any theory of the origin of directing effects by experiments on aromatic substitution; and I regret that I ever expressed a different opinion on that point. Naturally if the investigated consequences of a theory can be inverted by a change of emphasis, that theory cannot be tested. It remains true, however, that such experiments can *yield information* about the origin of directive power, and may form a *check* on any theory sufficiently precise to be so treated".

Robinson (1925) replied in September, again denying that he had altered his views but admitting that he "had found it necessary to adjust the earlier view of the parts played by conjugation and the general electrical effect respectively". This distinction was one Ingold was unable to see and he replied, briefly to that effect. The continuous barrage of letters debating intricate points of theory and detailed experimental results was completely incomprehensible to many chemists and even to most of those who had some interest in the subjects under discussion. Even the participants had trouble understanding each other and Armstrong's patience ran out about the middle of October when he tabled a motion at a meeting of the Council of the Chemical Society, in his own inimitable style: "that henceforth the absurd game of chemical noughts and crosses be taboo within the Society's precincts and that, following the practice of the press in ending a correspondence, it be an instruction to the officers to give notice 'That no further contributions to the mystics of polarity will be received, considered or printed by the Society". His challenge was not accepted because no one would second the motion. Nevertheless, a stalemate had been reached and there was a lull in the activities of the antagonists as 1925 drew to a close. Armstrong had never had any time for electronic interpretations of organic chemistry "No one", he said, "would hang him in chains, conjugated or otherwise" He dismissed Robinson's curved arrow with "A bent arrow never hit the mark" (Robinson, 1958).

The stalemate was broken when Robinson (1926a) announced new experimental results (Robinson and Ing,1926) on the nitration of benzylamines, where *meta,meta'*-dinitrobenzylamine was obtained from benzylamine - exactly the opposite result to that reported by Holmes and Ingold (1925). Other experiments showed conclusively that amine salts gave *meta* products and free amines gave *ortho/para* products. These results were clearly those predicted by Robinson on the basis of the alternate polarity theory and contrary to Flürscheim's alternate affinity theory. Now it was Ingold's turn to wriggle and he did so in his reply (Ingold, 1926a): "This paper, being a preliminary survey of a wide field ... described in each case the main product and by-products *isolated*; it was not stated that nothing else was *present*". Also, "It is true that our results were described as consistent with Flürscheim's theory, but this does not mean they are inconsistent with *general* polarity - the only form of polarity recognized by that theory". Flürscheim also wrote in the same issue, with more detailed argument and an account of experiments in progress with Holmes, who was now working with him. He got in one last jab with, "In view of Professor Robinson's many conflicting statements...it is scarcely surprising that, whereas Holmes and Ingolds' results (1925) contributed important and fresh evidence in favour of alternate polarities and a wonderful contribution to the same, Professor Robinson's partly conflicting results are held to be a confirmation of the same theory". Nevertheless, the game was clearly over when Ingold confirmed that his work with Holmes had been in error and that Robinson's results were correct. No doubt the fact that Ingold had published 34 papers in 36 months meant that his supervision of laboratory work was not as close as turned out to be necessary. At this point the Editor of *Chemistry and Industry* closed the correspondence column to this topic with the statement "Discussion of alternate polarities and kindred topics is of great importance, and we hope the letters we have published in these columns have cleared away a considerable mass of misapprehensions. We are, however, obliged to wait for a period before dealing with the subject again; a proportion of our readers fail to understand the whole arguments without a mental effort which is made unwillingly".

The hope expressed was a long shot because surely in most readers' minds "the considerable mass of misapprehensions" had completely swamped them and the mental effort required to clear it away was not so much made unwillingly as entirely beyond their abilities. However, one person had the will and the ability to profit from it, and that person was Ingold.

Ingold had received from Robinson manuscript copies of some of the latter's papers and he returned them with a seven-page covering letter. The letter begins "I return your papers. Thank you for letting me see them. They represent in my opinion a very fine effort, especially on the theoretical side, and the theory is certainly one of Organic Chemistry and not of aromatic substitution only". At the bottom of page 5 of his letter, Ingold says "I am glad to hear your benzylamine paper is shortly to be published. I shall also publish again on the subject but am not ready just yet. When I do I shall turn right around. I do not care two straws what the public in general...". Robinson lost page 6 of this letter, but its absence does not prevent the conclusion that Ingold had now abandoned Flürscheim's theory in favour of an electronic view of organic chemistry.

Chemistry and Industry continued to report Chemical Society Proceedings and on March 18 recorded Robinson and Ing's (1926) paper on the nitration of benzylamines. At the meeting Ingold said "that his view, which agreed in principle points with Robinson, was based on accurate determinations by Dr. Goss ...the ion directed *meta* to the extent of more than 80% and the uncharged molecule almost wholly *ortho/para*". At a subsequent meeting (May 6, 1926), Ingold presented six different papers including "The nature of the alternating effect in carbon chains. "Parts IV, V, VI and VII (Ingold 1926c). It was part V (Ingold and Ingold, 1926) with Hilda Ingold, which most clearly set his path by applying a single set of principles to different types of organic reactions. For aromatic substitution he talked about "electronic strain" transmitted from the

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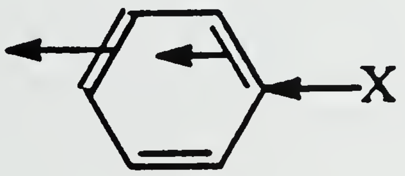

(*op*-substitution)

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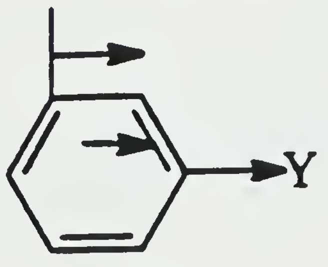


(*m*-substitution)

directing group through the benzene ring resulting in either a relaxation or constraint upon electrons, without actual transference.

"(A) represents an electronic interpretation of Flürscheim's mechanism for the development of partial valencies, and the strain directions correspond with Robinson's electron-transference paths...This combination of postulates appears to retain all the advantages and avoids the more obvious inconsistences and difficulties of the theories recently published and discussed". In other words this was a new theory, i.e. Ingold's theory. It may be noted that he avoided using curved arrows although he had previously used them in private correspondence with Robinson and in published papers.

Robinson was not present at this meeting but sent a communication noting that by courtesy of Professor Ingold he had had the opportunity to read the papers presented. He wrote (Robinson, 1926b), "The views now adopted by Ingold and his collaborators differed in no fundamental respect from those already advanced at various times by Lapworth and himself. The advocacy of non-polar theories of alternation, so characteristic a feature of Parts I, II and III of this series, have now been abandoned and this development was welcomed". In reply, Ingold said "The authors preferred their own view....and that in formulating it much benefit had been derived from consideration both of Flürscheim's theory and Robinson's. Since many important principles of Flürscheim's theory had now been reproduced in electronic language, it could not be sweepingly described as having been abandoned" (Ingold, 1926d).

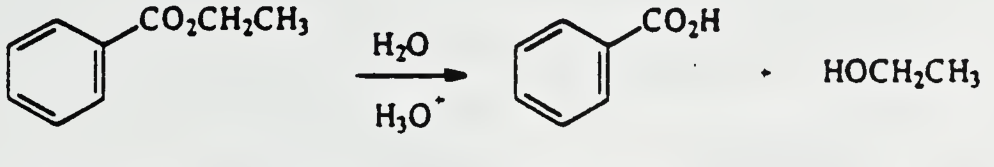
This essentially concluded the great debate on the theory of organic reactions. It was not an attack by Ingold on Robinson, nor was it an exercise in exchanging insults as some light-hearted accounts have alleged. It was a serious scientific debate in which the participants were seeking the truth. They occasionally became irritated with each other but in general they kept their tempers remarkably well. Robinson and Ingold, both changed their views in the light of their experiments and worked to find the correct theory which could be applied to all organic reactions. In fact, they were sorting out the two major effects of a substituent, called in modern terms inductive and resonance effects, and, at the same time, the structure and reactivity of electrophiles and nucleophiles. In this they were successful. However, whereas Robinson basically disliked argument and debate and was worn down by it, Ingold loved it and was vitalized by it. When the debate was over, Robinson went back to his natural product chemistry.

In contrast, Ingold continued to recast the electronic theory of organic chemistry in his own terms and supported it with a prolific flow of research papers. In 1926, he reversed the sign of the inductive effect of the methyl group from negative, as it had been denoted by Robinson, to positive. The reason he gave for this change was quintessential Ingold, "The electron releasing effect of the methyl group in toluene increases the electron density in the aromatic nucleus,and the differential coefficient of an increasing quantity is positive" (Shoppee, 1984).

In 1932 Ingold was forced to interrupt his research work because of problems with his eyes. The cure took the form of an extended visit to California where he wrote a review "Principles of electronic theory of organic reactions" (Ingold, 1934). This quickly became the seminal source on the theory of organic reactions for research workers on both sides of the Atlantic. From this source, Ingold's terminology and Robinson's curved arrows moved into the mainstream of chemical thought.

The assimilation of the electronic theory and curved arrows into the teaching of organic chemistry

An inspection of modern text books used for teaching introductory organic chemistry will reveal detailed multi-colour graphics of organic reaction mechanisms. An example is shown (without the colours) for the acid-catalyzed hydrolysis of an ester, Equation 10 and Fig 1[*], from the book currently used at Dalhousie University (Ege, 1994).



(10)

One of the reasons that this text was selected is the clarity of the schemes: complete Lewis structures with all lone pairs of each intermediate in the step-by-step mechanism and careful use of curved arrows to show all bonds formed and broken. As well, there is continuous qualitative discussion of reaction rate as a function of the structure of both substrates and reagents. In good modern textbooks (Carey, 1992), curved arrows are introduced as soon as possible. For reactions; "*Curved arrow notation* is used to show the electron pair of the base abstracting a proton from the acid. The *pair* of electrons in the H-A bond becomes an unshared pair in the anion, :A^-[†]. Curved arrows track electron movement, not atomic movement." (p 124).

For resonance; "What we try to show by the resonance formulation of ozone is the delocalization of the lone-pair electrons of one oxygen and the electrons in the double bond over the three atoms of the molecule. Organic chemists often use curved arrows to show this electron delocalization" (p 24).

The pedagogical reason for this approach is obvious. With practice, students do not have to rely entirely on memorizing reactions and mechanisms. They learn to predict what steps make chemical sense by a logical understanding of acids, bases, electrophiles, nucleophiles and formal charges. This approach attempts to minimize the problem as expressed by Conant in 1928 (Wheeler and Wheeler, 1982): "It is generally conceded that in order to teach any subject effectively, it is first necessary to arouse an interest in it. This is not easy in the elementary course in organic chemistry because of the bewildering array of facts and formulae which confront the beginner."

Conversion of text books to this modern standard was a slow process after the development of the concepts in the 1920's and 30's. Probable reasons for this delay were both the expense and difficulty, until recently, of producing graphics of this quality. However, the major reason was surely intellectual. Many years were required to convince academics that the ideas of the electronic theory were essential to understanding organic chemistry even at the research level, let alone at the introductory undergraduate level.

An interesting observation is the contrast in the use of curved arrows to describe resonance forms (electron density distribution in a molecule) as opposed to reaction steps (making and breaking of bonds). The first curved arrows used (Kermack and Robinson, 1922) describe how resonance in a triene leads to reaction by addition at the terminal ends. By 1926, Robinson was systematically using curved arrows for reaction steps. For instance, his description of the mechanism for the bromination of anisole is reproduced below where the arrows, labelled a, represent the first step

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[*] Reproduced with the permission of D.G. Heath & Co., Toronto.

[†] Note equation 1.

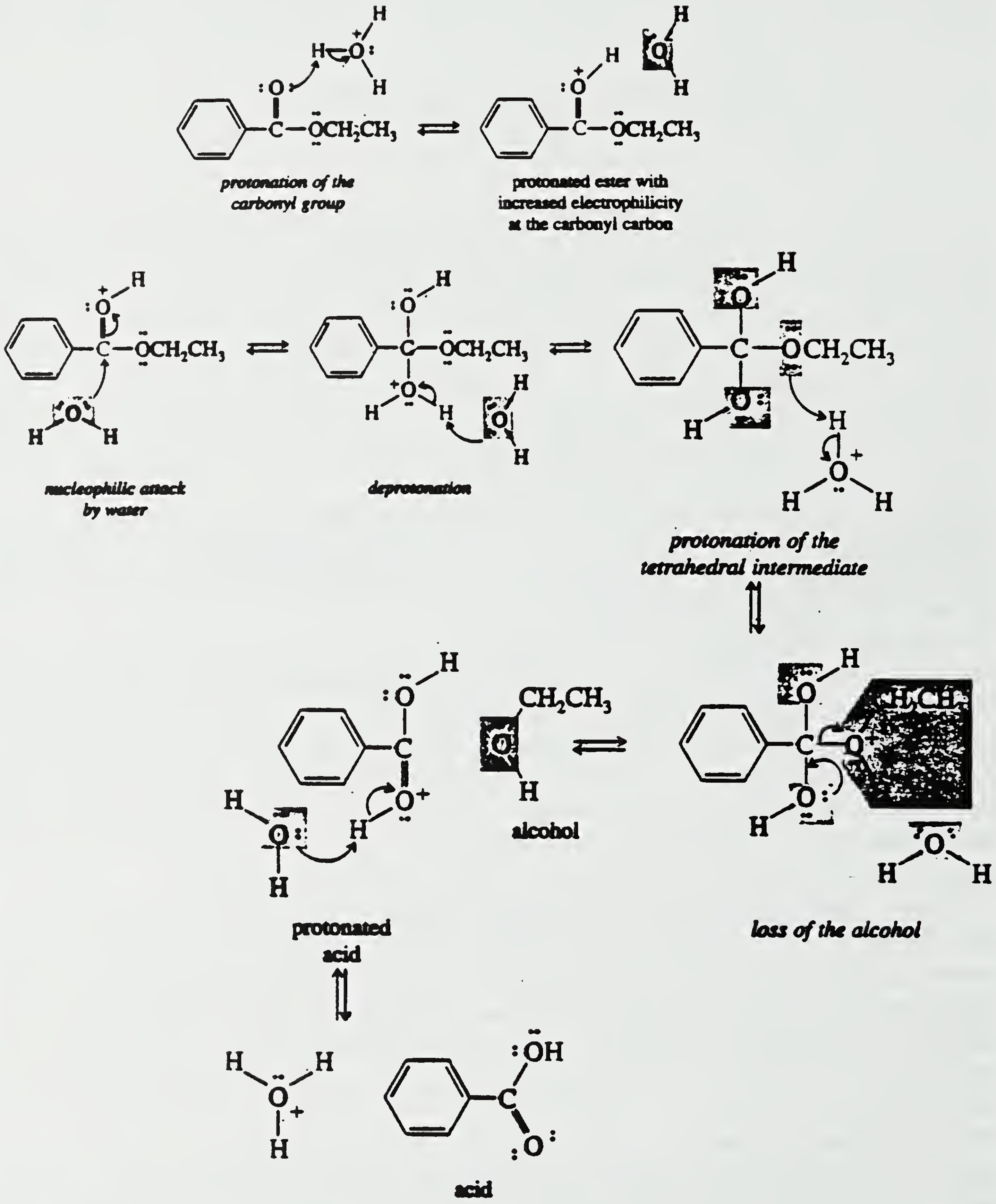
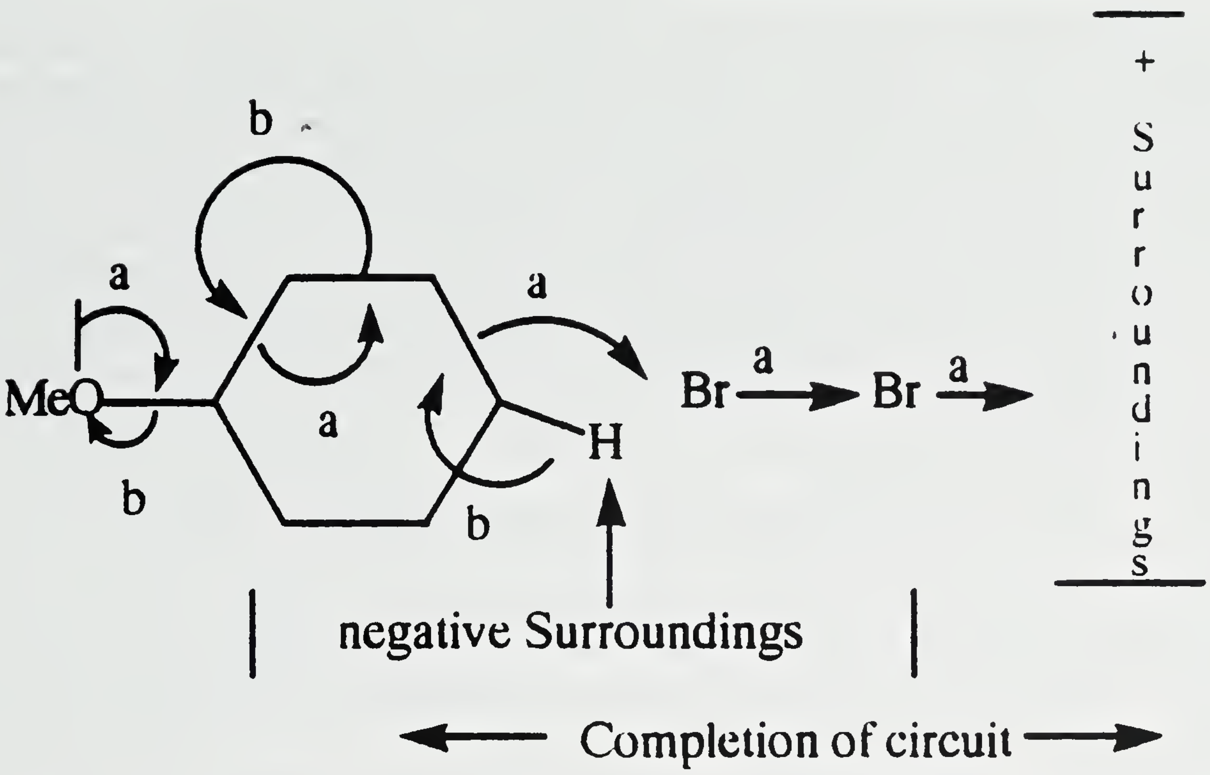

protonation of the carbonyl group
protonated ester with increased electrophilicity at the carbonyl carbon
nucleophilic attack by water
deprotonation
protonation of the tetrahedral intermediate
loss of the alcohol
alcohol
protonated acid
acid

Fig 1 Acid-catalyzed Hydrolysis of an Ester.



showing the *para* directing ability of a CH_3O group (the vertical line on the oxygen represents a lone pair of electrons) (Robinson, 1941 citing *Chem. News*, 133: 3455; 1926). The arrows, labelled b, describe the second step, deprotonation at the *para* position. The diagram provides an interesting analogy to an electrical circuit.

By contrast, in his 1934 review, Ingold only uses curved arrows for the interconversion of resonance forms. In 1953, Ingold wrote his definitive text (as a consequence of the Baker Lectureship at Cornell University in the fall of 1950) summarizing the advances in the electronic theory over some thirty years of his scientific career. Even by that time, he used curved arrows to describe reaction steps only sporadically. In the modern introductory text examples used above, Ege uses curved arrows for reaction steps, but not for resonance forms, and Carey uses them for both.

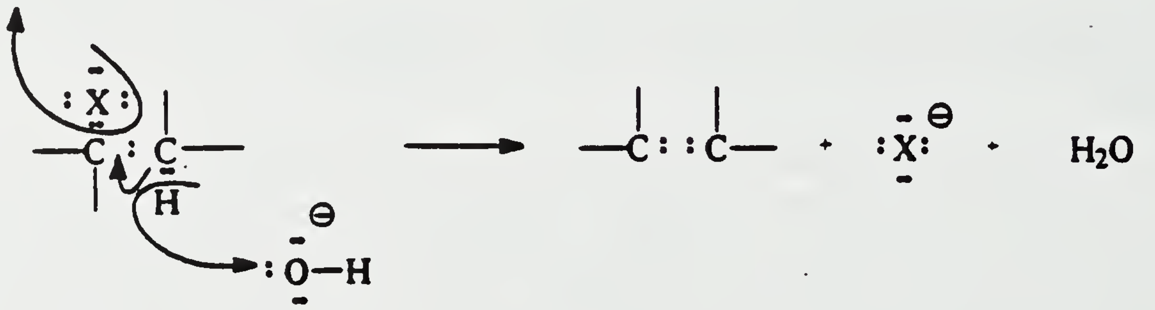
The original ideas of the electronic theory led to the development of the subject specialty called physical organic chemistry, first by Hammett (Hammett, 1940). Recent reviews (Saltzman, 1986; Tarbell and Tarbell, 1986; Brock, 1992) emphasize that although this subject developed from the ideas and research of the English school in the 1920's and 30's, the modern quantitative detail of physical organic chemistry was mainly American. The major thrust of organic chemistry in England continued to be synthesis and natural products, Ingold's and his collaborators' research groups being one exception.

Although research level books in both physical and synthetic organic chemistry finally began to include the electronic theory and the mechanistic approach, they did not adopt curved arrows. Thus, over a period of fifty years a series of physical organic books (Hammett, 1940, 1970, p 332; Remick, 1949, p 413; Alexander, 1950, p 224; Ingold, 1953, p 779; Gould, 1959, p 318; Hine, 1962, p 277; Wiberg, 1964, p 443; Lowry and Richardson, 1987, p 720; Carey and Sundberg, 1990, p 466) discusses the mechanism of ester hydrolysis using schemes devoid of curved arrows as well as the lone pairs required for Lewis structures. It is as if the "pure" physical organic chemists' approach was to avoid the descriptive crutch of the curved arrow. These books do use the curved arrow notation, at least sporadically, for other reactions and some (Remick, 1949; Alexander, 1950; Ingold, 1953) use them very consistently for resonance forms.

The conversion of introductory text books to the mechanistic/curved arrow approach was also a slow process. For instance, the book (Kipping and Kipping, originally Perkin and Kipping) used by the father of one of us (JAP) at the University of Manitoba in 1935 went through numerous editions, the first in 1894 and the last, more than fifty years later, in 1948. It used the classical approach which simply catalogued and described the numerous reactions of the various organic functional groups. As was traditionally done, it also separated aliphatic (Part I) from aromatic (Part II) chemistry. By this time, of course, Ingold had written his definitive review, but not a word of the theory is incorporated into this popular text book of the time. As described above, the effect of substituents in directing electrophilic aromatic substitution reactions was a key to the understanding of the electronic theory. The Kipping and Kipping text states very clearly (p. 384) that if one wants *p*-chloronitrobenzene, one must nitrate chlorobenzene, but to obtain *m*-chloronitrobenzene, chlorination of nitrobenzene is required. No explanation of the reasons for this is given although the importance of Crum Brown's empirical rule as an aid in remembering these and similar facts is stressed (p. 382).

In 1952, one of us (KTL) studied introductory organic chemistry at University College, London with E.D. Hughes, Ingold's long-time collaborator. The text used was Finar (Finar, 1951) and the use of curved arrows is introduced on page 15 for generation of two charge separated resonance forms of carbon monoxide in a discussion of the polarity of the C-O bond. However, presentation of reaction mechanisms is limited and curved arrows seldom appear. In the section on aromatic chemistry, the electronic theory of substitution is briefly reviewed (p. 413-421) and curved arrows are used to show resonance effects of substituents. Some curved arrows are included in the discussion of ester hydrolysis (p. 148).

In 1962 Morrison and Boyd's (1959) textbook, which at one time commanded 90% of the American market, was used by the other of us (JAP) at the University of Manitoba. This book was heralded because it consistently used a mechanistic approach to the learning of organic chemistry. This learning process was hung on a framework of electronic theory and did not require committing a catalogue of organic reactions to memory. However, the book did not use curved arrows consistently to describe electron flow. For instance, in the first edition, curved arrows are used on p 113 for an E2 elimination reaction as reproduced in Equation 11 - the arrow on the right violates the electron movement convention, which was not defined. Another example (p. 117, Equation 12) may be compared with the currently accepted arrows in Equation 1 for a proton transfer step.



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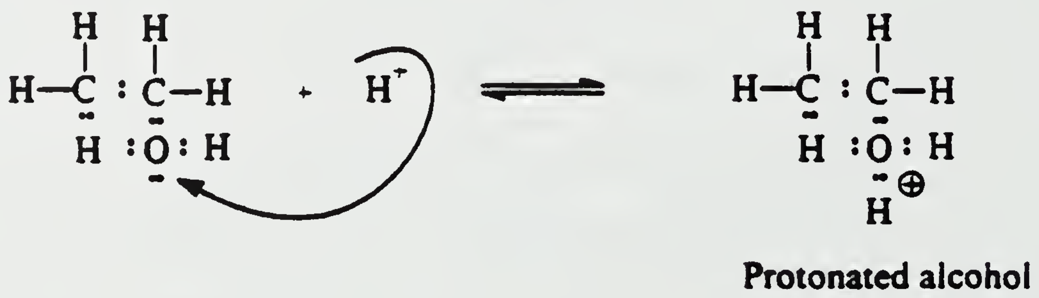
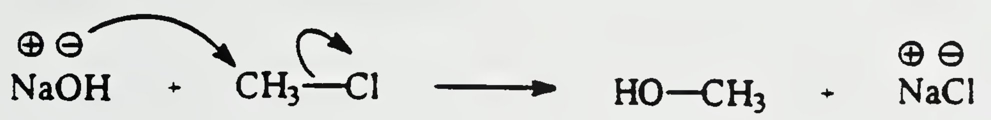


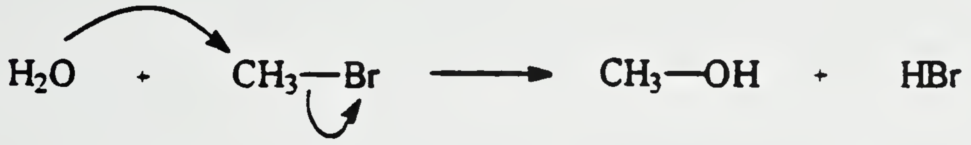
(12)

Another novel book (Cram and Hammond, 1959) emphasized reaction types grouped by mechanisms as opposed to the usual functional group approach, but the use of curved arrows was omitted for the generation of resonance forms. They were used for chemical reactions and were introduced for nucleophilic substitution on p. 199, "in order to indicate the direction of flow of electrons in equations, curved arrows are frequently added to the formulas". Surprisingly, the next sentence reads, "The arrows do not imply anything about the detailed mechanism of the reaction, which may be very complex". The S_N2 mechanism is shown (p. 199; Equation 13)



$\overset{\oplus\ominus}{NaOH}$
+
CH_3—Cl
HO—CH_3
+
$\overset{\oplus\ominus}{NaCl}$

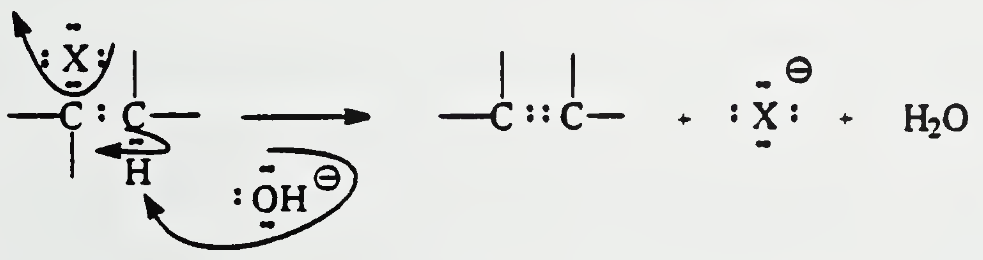
(13)



(14)

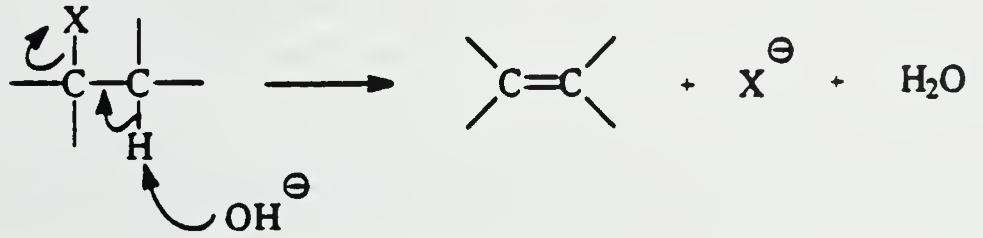
and later (p. 203), in a section on solvolysis reactions the conversion of methyl bromide to methyl alcohol is shown as reproduced in Equation 14. This is also an S_N2 reaction but is stated to be very slow because of the weakness of water as a nucleophile. The next two examples of solvolysis reactions in the book are S_N1 reactions involving a mechanism of rate-determining cation formation but no distinction is made at this point between the two mechanisms. In fact, the details (and Ingold's terms, S_N1 and S_N2) are only introduced in the next chapter and, again curved arrows are not used for reaction steps. Why the authors, who are internationally famous physical organic chemists, did not take the logical step of using curved arrows to describe the individual steps of these reaction mechanisms is not clear. The mechanism of acid catalysed ester hydrolysis is not given, though the initial protonation step is shown (p. 304), albeit without curved arrows.

In 1973, the introductory organic chemistry class at Dalhousie was using the third edition of Morrison and Boyd (1973). In this text curved arrows are first introduced on p. 157 in a rather elaborate style (Equation 15), which is similar to the style used in the



(15)

Represented as



where arrows show the direction of electron shift first edition (Equation 10), but now in accord with electron movement convention. The next equation in the text shows less elaborate arrows, as would be used in a current text, but with the lone pairs of electrons removed. The acid catalysed ester hydrolysis (p. 681) is still shown without arrows or lone pairs.

From 1975 until the present there has been a continuous stream of books, by more than a dozen authors, published for use in the standard full year (two semester) course in introductory organic chemistry. The standard of these books is uniformly high and selection of one by a given University is a difficult task. During this period, the quality of drawings, including the use of curved arrows and complete Lewis diagrams, has continuously evolved and improved. The mechanistic approach as derived from electronic theory is firmly entrenched.

Thus, a fundamental pedagogical tool in the modern approach to organic chemistry had a difficult and contentious birth, followed by a long and confused upbringing over a period of about 60 years, before it finally reached the sophisticated and elegant presentations exemplified by Fig 1 (Ege, 1994).

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A NEW TWO-DIMENSIONAL LATTICE OF COORDINATION NUMBER FIVE*

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B3H 3J5

An introduction is given to the symmetrical square and triangular two-dimensional Bravais lattices. Next the two most symmetric two-dimensional, non-Bravais regular lattices are introduced - the honeycomb and the kagome - each derived from the triangular lattice. These four lattices have coordination numbers of four, six, three and four respectively. A third high symmetry two dimensional non-Bravais regular lattice, also derived from the triangular lattice is introduced. The remarkable fact about this previously unknown lattice is that its coordination number is five.

On introduit des treillages Bravais symétriques qui sont carrés et triangulaires et qui ont deux dimensions. On présente ensuite des treillages non-Bravais réguliers les plus symétriques qui ont deux dimensions, le rayon de miel et le kagone, chacun desquels découle du treillage triangulaire. Ces quatre treillages ont des numéros de coordination de valeur quatre, six, trois et quatre respectivement. On présente enfin un treillage régulier, de haute symétrie, bidimensionné et non-Bravais, une dérivation du treillage triangulaire. L'aspect remarquable de ce treillage, préalablement inconnu, est le fait que cinq est son numéro de coordination.

Introduction

Bulk solid materials are most commonly found in a crystalline state. Their constituents - atoms, molecules or ions - typically occupy the sites or vertices of a three dimensional lattice. Familiar examples are the simple cubic, body centered cubic, face centered cubic, hexagonal close packed and diamond lattices. Descriptions and drawings of these lattices can be found in most textbooks on solid state physics.

Less familiar but not uncommon are two-dimensional lattices. Kittel (1956) provides an exceptionally good introduction to them. They may occur as monatomic layers well separated from one another within the structure of a complex three-dimensional crystal and these have become of greater interest since the discovery of high temperature superconductors (Bednorz and Müller, 1986). In typical high T_c cuprate superconductors such as $YBa_2Cu_3O_{6+x}$ or $La_{2-x}Sr_xCuO_2$ two-dimensional layers of CuO_2 within the parent compound appear to serve as the medium in which the current flows without resistance (Manousakis, 1991).

For many years it has been accepted that there are four regular two-dimensional lattices of high symmetry - the square, triangular, honeycomb and kagome; in each, all vertices are equivalent. In the four lattices each vertex has four, six, three or four nearest neighbour vertices or sites respectively. In this paper a previously unknown two-dimensional lattice of high symmetry in which all the vertices are equivalent and each vertex has five neighbours is presented.

Two Bravais lattices - square and triangular

A Bravais lattice is a simple lattice in which any two vertices are equivalent under translation alone (in more complex lattices two vertices are in general equivalent only under a combination of translation and rotation). The two most symmetric and common two dimensional Bravais lattices are the square (Fig 1a) and triangular (Fig 1b).

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* Dedicated to the memory of Walter J. Chute

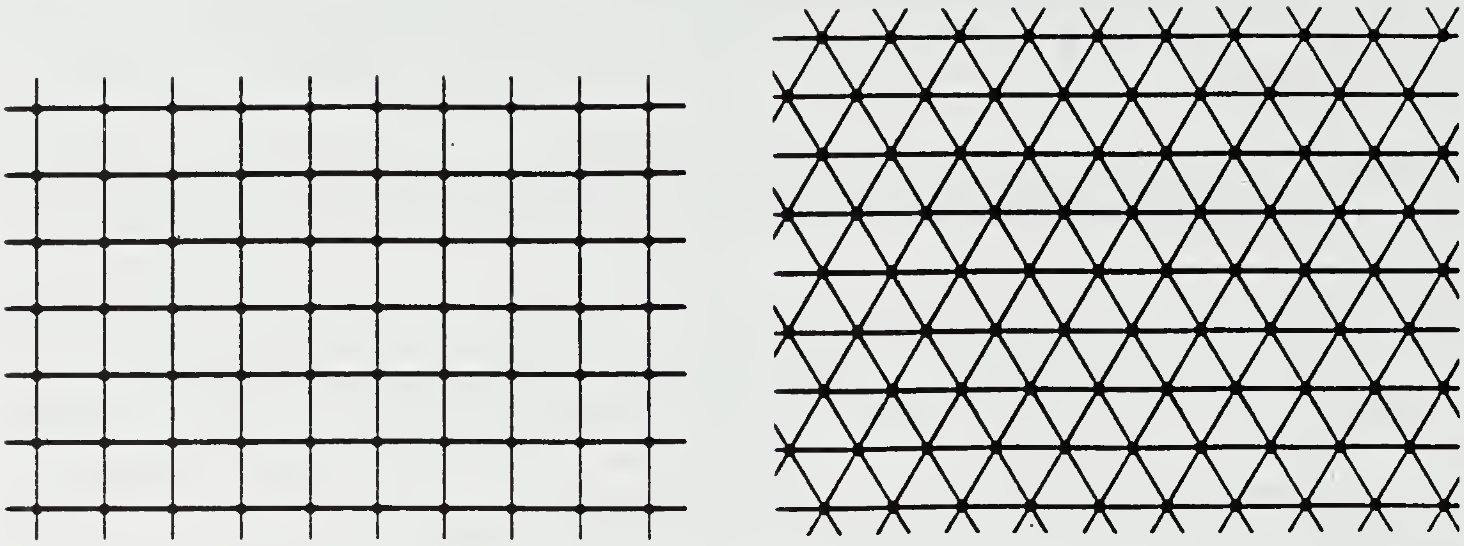

Fig 1 The two most symmetric two-dimensional Bravais lattices (a) the square lattice; (b) the triangular lattice.

Suppose you were a small but highly intelligent insect. You could walk along the nearest neighbour bonds from one vertex of a Bravais lattice to any other vertex and your view, facing the same direction, would be identical to that from the first vertex. This is a demonstration of the translational invariance of a Bravais lattice.

Next suppose that you, the intelligent insect, remain at the same vertex of the square lattice but you rotate counter-clockwise by 90°, 180° or 270°, your view does not change at any of the four positions. This demonstrates the rotational invariance of the square lattice. Similarly rotation by 60°, 120°, 180°, 240° or 300° at a vertex of a triangular lattice provides an identical view. Thus the square lattice has fourfold rotational invariance and the triangular lattice has sixfold rotational invariance. This rotational invariance is intimately related to the fact that a vertex on the square lattice has four nearest neighbours (coordination number four) while a vertex on the triangular lattice has coordination number six.

These two lattices each have a third type of invariance. If several fellow insects erected a large mirror perpendicular to the lattice plane and orientated along one of the nearest neighbour bonds the reflection in the mirror would be identical to the view before it was obscured by the mirror. This is reflection invariance.

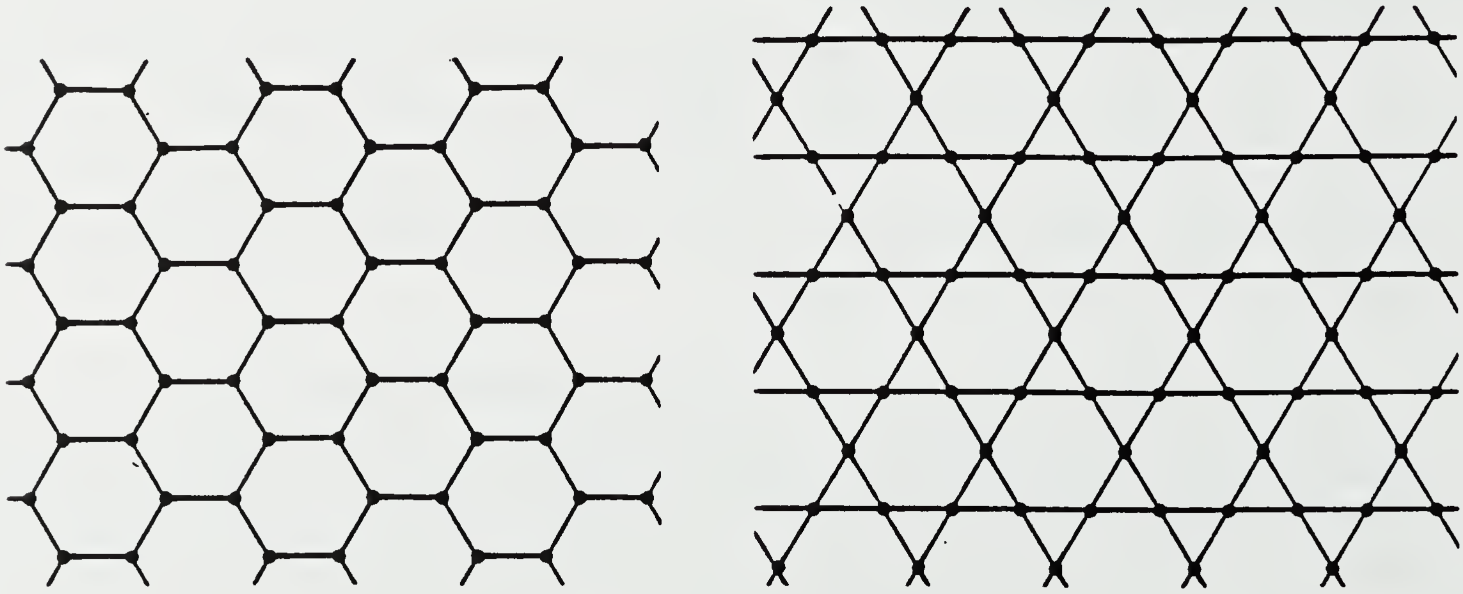

Fig 2 The two most symmetric two-dimensional non-Bravais lattices (a) the honeycomb lattice; (b) the kagome lattice.

Two non-Bravais lattices - the honeycomb and the kagome

The two most symmetric two-dimensional non-Bravais lattices are the honeycomb and the kagome lattices illustrated in Fig 2a and Fig 2b respectively. Note that the honeycomb lattice has coordination number 3 and that the kagome lattice has coordination number four. Now if you, bright insect, standing on the honeycomb lattice, walk from your initial vertex to one of its three nearest neighbour vertices without turning, the appearance of the lattice in front of you appears different. However, if you also turn through 60°, 180° or 300° at the second vertex the lattice looks the same as you saw it initially. This is a manifestation of the invariance of the honeycomb lattice under a combination of translation and rotation.

If you are standing on a vertex of the kagome lattice and walk to the next vertex you must also turn through an angle of 60° or 240°, clockwise or counter-clockwise depending on which vertex you walked to, for the lattice scenery to remain the same. This is a second example of a non-Brevais lattice being invariant under a combination of translation and rotation. If you now stand at the center of one of the hexagon "holes", the lattice from this vantage point is more symmetric for it is invariant under rotation about 60°, 120°, 180°, 240° or 300°. That is, it has sixfold rotational invariance. The honeycomb lattice also has reflection invariance in any mirror erected to run through the center of a hexagon and any one of the 6 vertices nearest to the center. The kagome lattice has the same reflection and sixfold rotation invariances when observed from the center of one of its hexagons.

The reader may observe in Fig 2a that the honeycomb lattice is invariant under translation if the translation is to a *second-nearest* neighbour of the initial vertex. Similarly, an examination of Fig 2b reveals that the kagome lattice is invariant under translation if the translation is to a third-nearest neighbour.

Construction of other lattices from the triangular lattice

Fig 3a demonstrates that the triangular lattice may be considered as consisting of 3 identical triangular sublattices whose vertices are labelled A, B, and C. Deleting any one of three sublattices produces a honeycomb lattice consisting of two triangular sublattices as illustrated in Fig 3b. The triangular lattice may be considered also as consisting of four identical triangular sublattices. Eliminating any one of the four sublattices yields the kagome lattice. Thus the kagome lattice consists of 3 triangular sublattices.

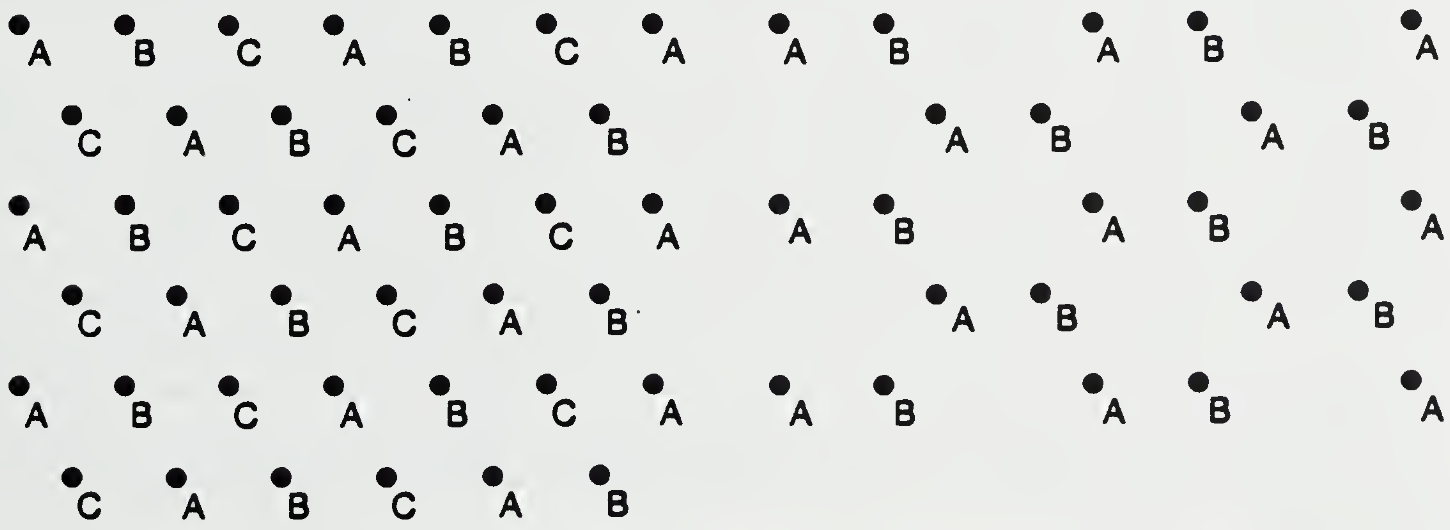

Fig 3 Deriving the honeycomb lattice from the triangular lattice (a) the triangular lattice as composed of three triangular sublattices A, B, and C; (b) the honeycomb lattice as composed of triangular sublattices A and B.

The triangular lattice can not be considered as consisting of two, five, six or eight triangular sublattices, but it can be considered as consisting of *seven* equivalent triangular sublattices as shown in Fig 4a. To make this comparison more obvious the vertices of sublattice G have been joined by solid lines. If any one of the seven sublattices is eliminated (e.g. G) a lattice of coordination number five, illustrated in Fig 4b, is obtained. It is like the honeycomb and kagome lattices in containing an array of hexagonal "holes" created by the deletion of one of the triangular sublattices.

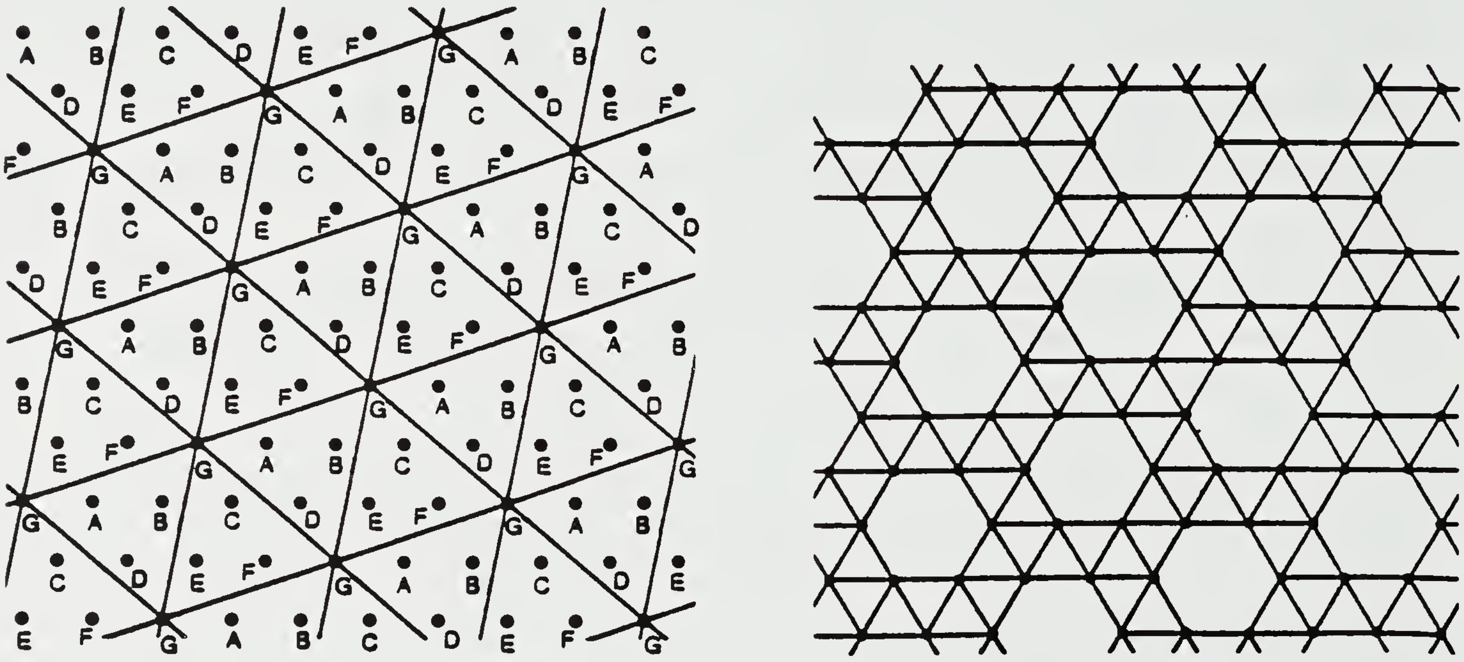

Fig 4 Deriving the new lattice from the triangular lattice (a) the triangular lattice as composed of seven triangular sublattices A, B, C, D, E, F, and G; (b) the coordination five lattice as composed of all but one of the seven sublattices in Fig 4a.

Consider the symmetry of this new lattice; seen from the center of one of the hexagons it is, like the honeycomb and kagome lattices, invariant under rotation through a multiple of 60°. However, unlike the other two non-Bravais lattices, the new lattice does not have invariance under reflection in any line through the center of a hexagon. Indeed it does not have reflection invariance in any straight line drawn anywhere on the lattice. What is important for any lattice is its symmetry with respect to the vertices of the lattice.

To facilitate understanding of the translation-rotation invariance of the new coordination five lattice, it has been decorated in Fig 5 with stylized maple leaves.

Each vertex is now directly underneath the point on the leaf where its veins meet, the leaf's vertex. Pick any leaf as central, and notice that each of its five nearest neighbours is orientated at 60°, 120°, 180°, 240° and 300° with respect to it. Suppose once more that you are an intelligent insect. What the leaves are telling you, for example, is that if you are initially standing on the vertex of a leaf facing the end point of the leaf and walk in that direction to the vertex of the next leaf, you must then turn counter-clockwise by 120° for the lattice with all its leaves to look identical to its appearance when you left your starting point. If you walk to any one of the other four nearest leaves then the angle you must rotate through for the lattice to appear unchanged is one of 60°, 180°, 240° or 300° depending on which neighbouring leaf you reach.

The translation-rotation invariance relation between any pair of nearest neighbour vertices means that this coordination five lattice is regular and has point group symmetry 6 and space group symmetry p6 (Kittel, 1956).

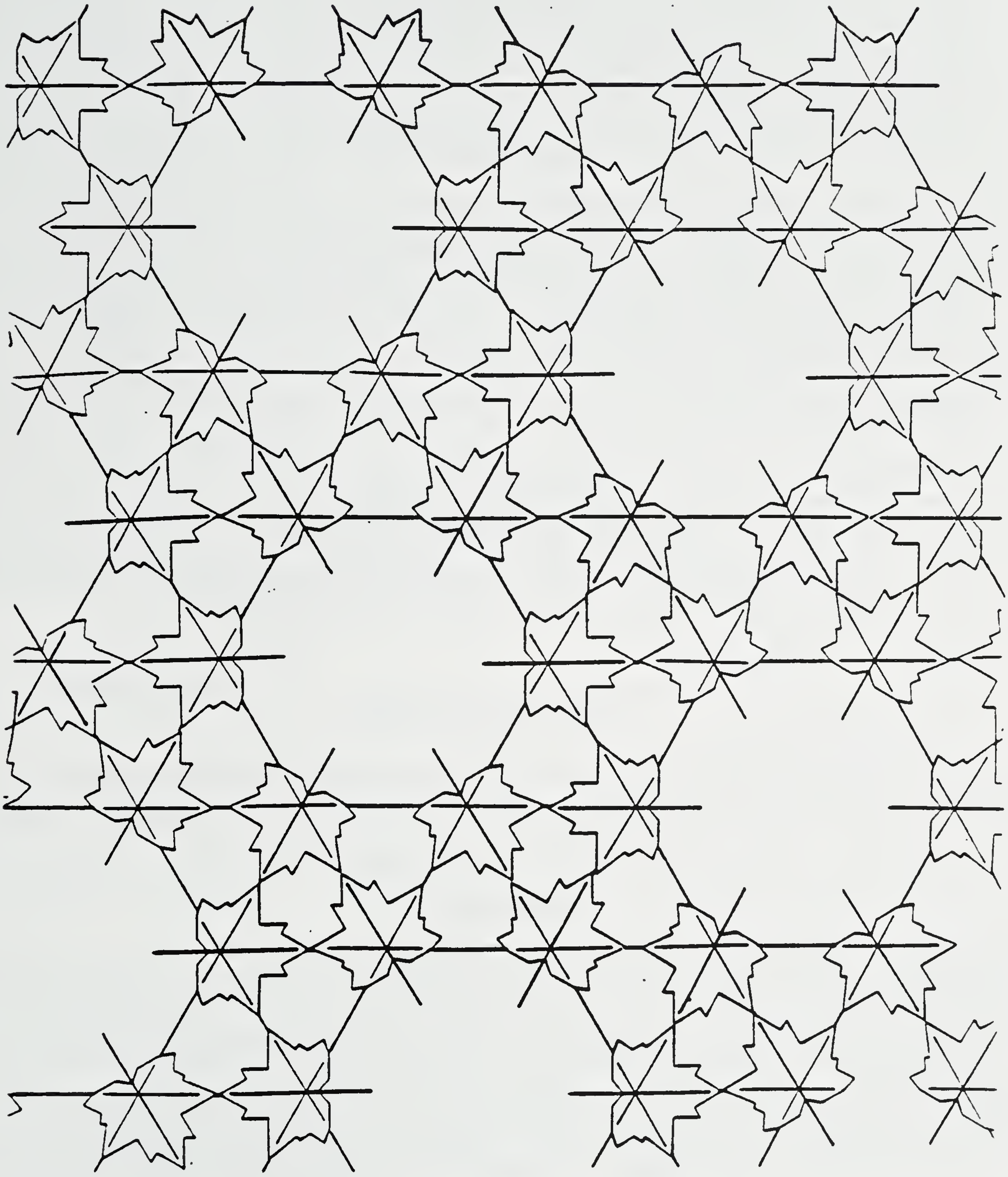

Fig 5 The new lattices decorated with stylized maple leaves. All leaves on each sublattice point in the same direction, but the leaf orientations on different sublattices differ in direction by a multiple of 60°.

Discussion

In this paper it has been shown that the honeycomb (Fig 2a) and kagome (Fig 2b) lattices can be derived from the triangular lattice by deleting sublattices. This led to the derivation of a new lattice by first regarding the triangular lattice as being composed of 7 sublattices and then deleting one of the latter. The remarkable feature of this new lattice is that it is a regular lattice with coordination number five. The symmetry of this lattice has been described in part by decorating the lattice vertices with stylized maple leaves oriented in a different direction on each sublattice. This lattice has half the symmetry of its triangular parent and three quarters the symmetry of the square lattice.

One may predict that real physical systems having the structure of the coordination five lattice will be found and/or made. The physical properties of such systems are likely to be of interest and two possible examples in the field of magnetism are worth mentioning. The Ising model of nonconducting ferromagnets or antiferromagnets is the only physically realistic model of magnetic systems whose properties have been calculated exactly on two-dimensional lattices (the properties of several magnetic models have been calculated exactly on one dimensional lattices or chains). These exact solutions (Baxter, 1982) are of interest to theorists and experimentalists and it can be expected that the Ising model will be solved exactly on the coordination five lattice.

The properties of other magnetic models e.g. the Heisenberg model, that are somewhat more complex than the Ising model, have not been calculated exactly on any two-dimensional lattice, and it appears unlikely that exact solutions will be found in the near future. However, several different methods have been developed to calculate the properties of such models to high precision on standard lattices and the same methods are applicable to these models on the new lattice.

Acknowledgements

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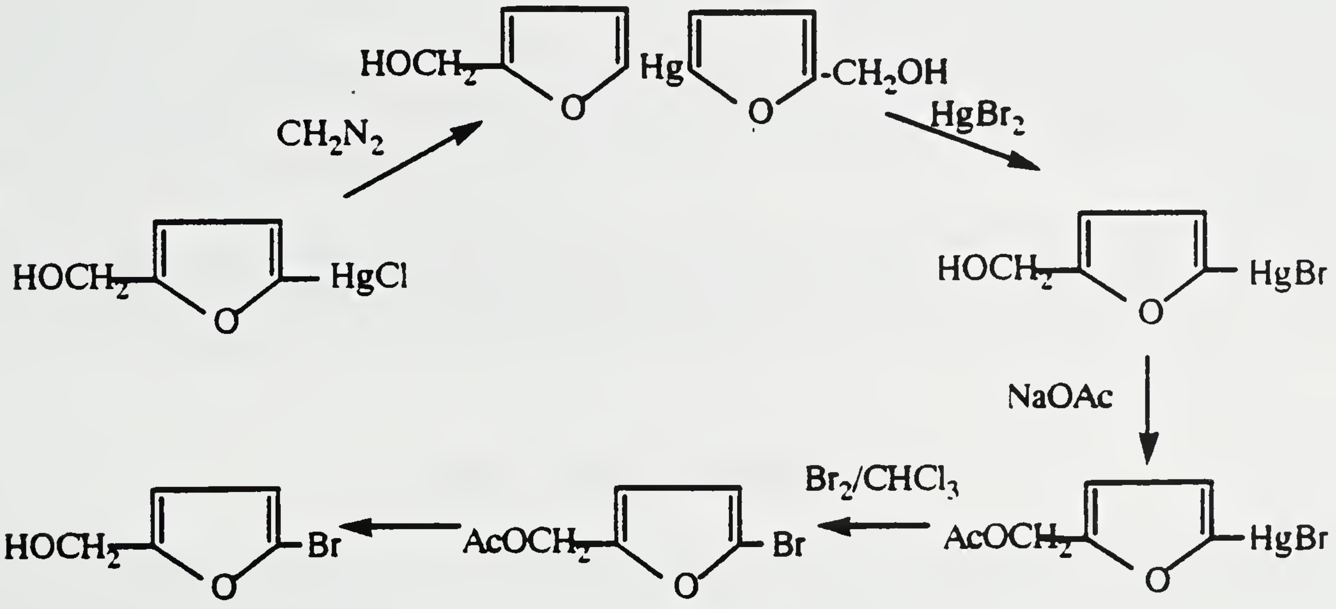
Obituary of W.J. Chute

Dr. Walter John Chute, (Secretary, 1945-1948; President 1952-1954) who died on 9th December 1991 was born on the 28th March 1914 at Brooklyn Corner, Kings County, Nova Scotia. He graduated from King's County Academy in 1931 with an average of 77% in mathematics, physics and chemistry and proceeded to the Teacher's Training College in Truro, where he was awarded a "Superior First" diploma in 1934. He then taught science in High School for a few years but was persuaded to read chemistry at Acadia University, probably by Chester Small. He graduated with honours in chemistry in 1939; unfortunately records of that time at Acadia University have disappeared.

He was appointed as a laboratory assistant by Toronto University and he began work for the M.A. degree under the supervision of G.F. Wright. Wright had been a student of Gilman and at that time he was continuing work on the displacement reactions of chloromercury derivatives; which in Chute's hands led to practical syntheses of 5-bromo, 5-iodo and 5-methylfurfuryl alcohols. 5-Bromofurfuryl alcohol, an unstable compound was prepared in good overall yield by the interesting reactions shown below.



The dechlorination with diazomethane is unusual and the selective esterification of the primary alcohol without displacement of the organometallic moiety is noteworthy. This work also led to the preparation of 5-chlorofurfural in 33% yield on the 96 g scale, by the demonstration that the reaction was catalysed in the presence of benzoyl peroxide and sulfur. This reactive compound has been used as a starting material and as an intermediate in the synthesis of many natural products. For this work Chute was awarded the M.A. degree in 1940.

He continued in Toronto with Wright as a National Research Council Scholar, to study for a Ph.D. degree, but the work was necessarily constrained by wartime conditions. The superior (to TNT) handling and explosive force of tri-N-nitrohexahydrotriazine (RDX) had ben discovered in 1899 by Henning (Ger. Patent 104280; see also Hale, *J. Amer. Chem. Soc.*, 47: 2754). The explosive was prepared by nitration of hexamethylenetetramine and Wright undertook a study of the byproducts of the reaction, the general reaction of secondary aliphatic amines with nitric acid and the mechanism of the dehydration of the nitrates initially formed. Chute's part in this relatively large and important programme lay in the investigation of the preparation of nitramines from secondary aliphatic amines. He showed that the dehydration of nitrates was catalysed by chloride and that nitramines ($R_2N.NO_2$) could also be obtained by oxidation of nitrosamines ($R_2N.NO$) with persulfate at 25°; a procedure that might incur displeasure in administrative circles these days. This work led to the preparation of dinitroxydiethylnitramine ($(O_2NO.CH_2CH_2)_2N.NO_2$), an explosive having similar power to RDX, on the 823 g scale, the procedure involving mixing 1.6 kg of nitric acid with 2.75 kg acetic anhydride!. Chute demonstrated that most of the acetic acid byproduct of the reaction and the acetone used, could be recovered giving a final cost (excluding energy and depreciation) of 23¢ lb^{-1} (in 1943; Ph.D. thesis, University of Toronto).

In 1943 Chute was appointed as an associate professor in the Chemistry Department at Dalhousie University and for the next 36 years returned to his original vocation; teaching undergraduate organic chemistry. He became Head of the Department in 1954 and remained in that position whilst the graduate program was expanded mostly by the initiation of a Ph.D. program with the help of A.C. Neish and the National Research Council.

Chute retired in 1979 but continued to teach in the Department and these activities resulted in some interesting research closely related to that done 30 years earlier in Toronto. An undergraduate raised the question of the structure of the aldehyde bisulphite compounds which at that time were thought (Beilstein) to have the structure $R.CH(OH)\text{-}O\text{-}SO_2H$ by contrast to the nitrogen analogues ($R.CH(OH)\text{-}NH_2$). Chute prepared crystalline $HO.CH_2SO_3K$ and X-ray crystallography showed S-C bonding; hence the formula accepted for these compounds for more than 100 years was incorrect.

Walter Chute was a benevolent teacher and an understanding colleague who has been deservedly honoured by the Dalhousie University Chute graduate student award and the Chute lectureship, both of which have brought talented chemists to Halifax.

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